Steven M. Ruben
Appl. No. 10/662,429

Department Mol. Bio.
Subject 9/15/95 - 11/29/95
Name ANN KIM # 11
Address

National Brand
Computation Notebook
11 3/4" x 9 1/4", 4 x 4 Quad., 75 Sheets 43-648

0 73333 43648 8

AVERY DENNISON
Office Products
Chicopee, MA 01022

Ruben EXHIBIT #94

Department Mol. Bio.
Subject 9/15/95 - 11/29/95
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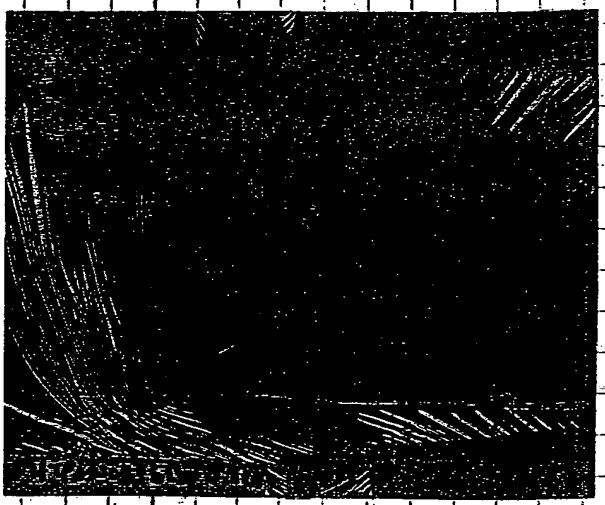
Ruben EXHIBIT 2094
Ruben v. Wiley et al.
Interference No. 105,077
RX 2094

(See Pg 152 Book #10 → Lab Notebook # 345)

9/15/95

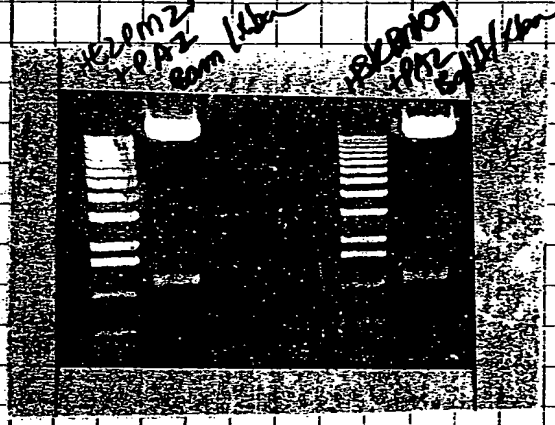
Wash Blot 1XPBS.
5 min at RT w/ Shaking
Add 2° Ab at 1:2000
in PBS
Incubate at RT for 1 hour with Shaking
Rinse in 1X PBS
Wash 5 min at RT with 1X PBS
Rinse in 50mM $Na_2H_2PO_4$ pH 7.
5 min at RT
Add Substrate:
12.5 ml 50mM $Na_2H_2PO_4$ pH 7
25 mg BNADH
250 µl phenol
8.5 µl 30% H_2O_2
0.375 ml NBT (10 mg/ml)
Incubate at RT until Colors develop.
Stop Reaction with dH_2O



Freeze Os Cells
Split 1 flask 1:40

9/22/95

Run 10ul on gel w/ 1Kb ladder



Looks good

Submit for Sequencing

HEEPM21+PA12.	HSK6N09+PA12
RP01	RP01
RP15	RP07
RP16	RP08
RP18	RP81
RP81	FP04
FP02	FP06
FP11	FP80
FP14	
FP17	
FP80	

Ken M Rich
9/24/95

9/25/95

Transfection of Lac Z /β-gal in 100 ug/ml & 150 ug/ml DEAE-Dextran

Remove Supernatant from Cells
Wash Cells 1X PBS 5min at RT
Fix Cells 3.7% Formaldehyde in PBS
Incubate at RT 30min
Remove Fix
Wash Cells 2X PBS 5 min at Room temp

9/25/95

Submit HE2PM21 +PA2
to protein Expression.

Set-up Cells – Cos Cells
for transfections
6 Well plates
1.5×10^5 cells / well

Clean-up DNA for transfections

HTPAN08S04 pCDNA
pCDNA 3'HA
pCDNA 5'HA

HT4SB02 pCDNA
pCDNA 3HA
pCDNA 5'HA

2x Phenol:Sevag (1:24, IAA:Chloroform)
2x Chloroform

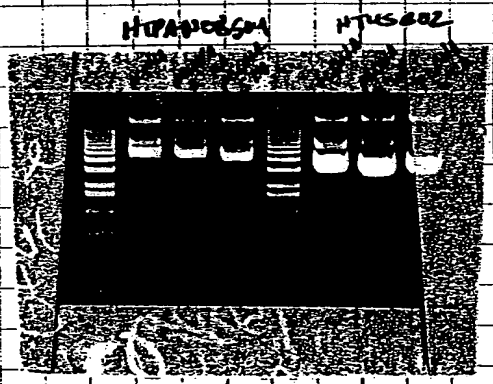
2x EtOH ppt.
Wash 70% ethanol – Let pellet Dry
Resuspend pellets in 100 µl TE

9/26/95

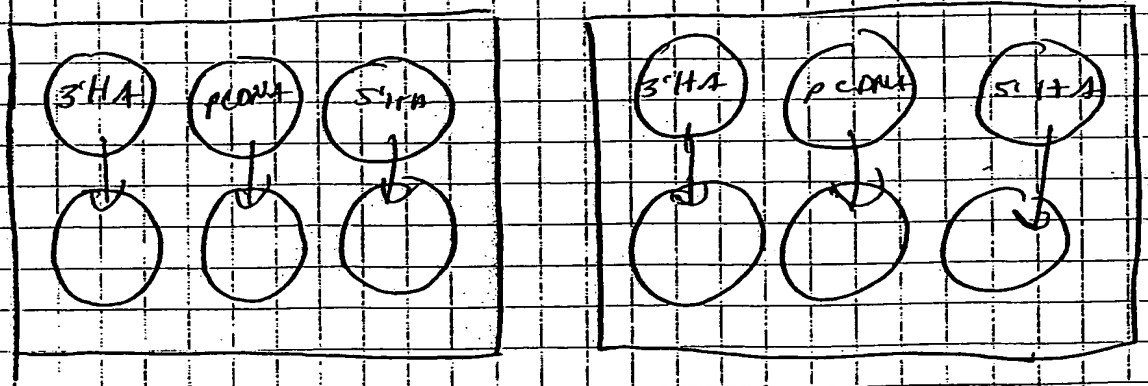
Dilute DNA 1:200 in H_2O
Read OD 260/280.

	260.0 nm	280.0 nm	
	280.0 nm	260.0 nm	
HTPA108 pCDNA	1.4833	0.6742	0.46 ug/ml
HTPA108 3'HA	1.4586	0.6885	0.40
HTPA108 5'HA	1.3024	0.7678	0.5
HTPA108 HT45B02	1.5920	0.6281	0.88
HT45B02 3'HA	1.4943	0.6692	0.91
HT45B02 5'HA	1.5087	0.6626	0.68

Set up transfection
Run 1ul on gel with
1Kb ladder



Set up transfections:
Use 100 ug/ml DEAE
Dextran
looks like it works the
Best from Lot 2 - Beta Gal Test



HTPA108 HT45B02

9/26/95

			5 µg
HT4SB02	pcDNA	0.88 µg/µl	5.68 µl
	pcDNA 3'HA	0.91 µg/µl	5.5 µl
	pcDNA 5'HA	0.68 µg/µl	7.35 µl
HTPANOX6S04	pcDNA	0.46 µg/µl	10.9 µl
	pcDNA 3'HA	0.4 µg/ml	12.5 µl
	pcDNA 5'	0.5 µg/ml	10.0 µl

Make 15 ml transfection Cocktail
- 1.5 ml Neutradurm
- 150 µl DEAE Dextran
- 150 µg Chloroquin 5 mg/ml
- Mix well

Add 1 ml transfection Cocktail to DNA
Mix well
Add to well.
Incubate 37°C / 5% CO_2 for 2 hrs.

Shock Cells
10% DMSO in cDMEM
2 min at RT

Remove Supernatant

9/28/95

TRANSFECTIONS –

HT4SB02 + PCDNA / 5'HA / 3'HA

HTPAN08 + PCDNA / 5'HA / 3'HA

Cells have been incubating at 37°C
5% CO_2 for 48 hrs ↓

Remove media
Wash Cells 1X PBS 5min.
Fix cells w/ 3% Paraformaldehyde.
in PBS.
1.5g paraformaldehyde
45 ml H_2O
12.5 ml 10N NaOH
Heat 40-50C
Add 5 ml 10X PBS.
Cool to RT
Fix for 30–35min at RT
Wash Cells 10 min RT with
PBS + 10mM Glycine
Incubate 5min – Time is Critical –
with 1% Triton X-100 in PBS.
Wash Cells 5 min RT with
PBS + 10mM Glycine
Incubat Cells 1½ hrs RT with
PBS + 25mM Glycine.

Block Cells in Antibody Binding Buffer.
20mM $NaHPO_4$ pH 7.0
0.5M NaCl
0.05% Tween-20
0.1% BSA
With 2% Goat Serum for 2 hrs at
Room Temp

9/28/95

Remove Blocking Buffer
Add 1° Antibody into Antibody
Binding Buffer + 2% Goat
Serum
Use HA TAG - SCP-12CA5-J
Purified Ab.
at 1:1000 dilution
Incubate at 4°C O/N

ENTERED Project information for:

HSRBN09
HT4SB02
HE8SH43
HE2PM21 → HDAATH602
into IRIS!

Split Cos Cells 1:40 into
T-75 flask

9/29/95

Remove 1° Ab
Wash Cells 2x - 10 min RT
with 10 mM Glycine in PBS
Remove Wash
Add 2° Ab 1:2500 RITC conjugated
Antibody in Ab Binding Buffer + 2%
Serum
Incubate RT 1 hr. w/ alum foil
Covered

9/29/95

Aspirate off 2° Ab
Rinse cells 1x 10mM Glycine + PBS
Aspirate off Rinse
Wash cells 2x - 2ml
10mM Glycine + PBS
Aspirate off Wash
Add 2 drops of photo mount
and cover w/ glass cover slip
View under flourescint microscope
Keep Covered in Alum. foil at
4°C.

Submit -
HE2PM21 + PA2
HSKBN09 + PA2
HE2PM21.
for Sequencing

10/2/95

Split Cos Cells
1:40 into T-75 flask
and plate out 2-6 well plates
for transfections
Seed plates at ~ 1×10^5 cells/well
Incubate 37°C 5% CO_2 O/N

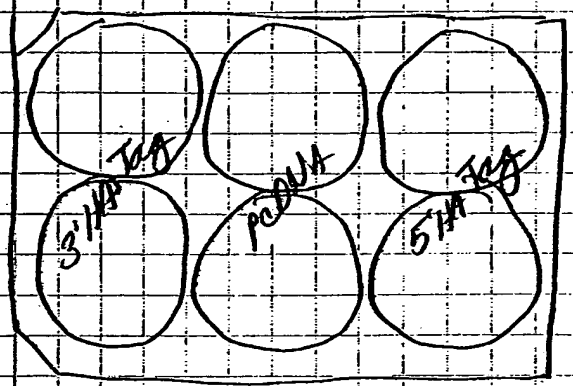
PCR New
HE2PM21 - PCR'ed insert
New 5' NCO I primer made

01/3/95

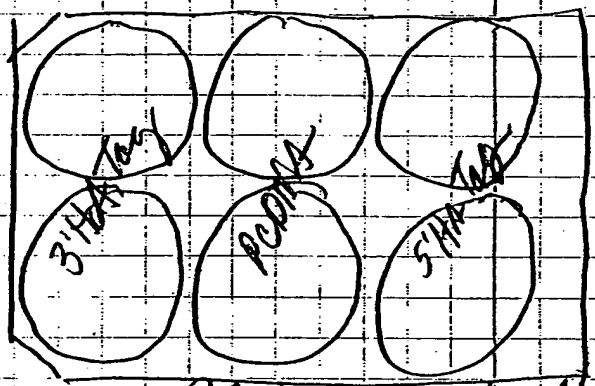
Transfections

		conc/ml	5ug
HT4SB02	pCDNA	0.88	5.68 ul
	pCDNA 5'HA	0.68	7.35 ul
	pCDNA 3'HA	0.91	5.5 ul
HTPAN08 5/4pATG	pCDNA	0.46	10.9 ul
	pCDNA 5'HA	0.5	10 ul
	pCDNA 3'HA	0.4	12.5 ul

Set-up Duplicates of each.



HT4SB02



HTPAN08 5/bpATG

Make 15 mls Transfection Cocktail

1.5 ml Neutradoma
150ul DEAE-Dextran (10mg/ml)
150ul Chloroquin (5mg/ml)
mix well.

Add 1ml to 5ug DNA & Pipet well
Add to well
Incubate 37°C 5% CO_2 for 2hrs.
Remove transfection Cocktail from wells

10/4/95

Inoculate 4ml LB + Amp / Kan:

HTPB411 + PQE60 - 3-4
HMSAF22 + PQE60 - #9
HTPAN08 518bp ATG + PDI0 - D9
" " " + " - D5
" " " + " C7
" " " + " A2

Inoculate 4ml LB + Kan:
M15 rep5 Cells

Inoculate from frozen Glycerol Stocks.

Incubate 37°C 5 hours -
till OD_{600} ~ 0.4-0.6

To 1000 µl LB + Amp Kan
+ transfer 500 µl of culture
To remaining Culture add 100mM
IPTG to 2mM - 80 µl
Incubate 4 hours
Store at RT O/N.

Alex 10/4/95

10/5/95

Run 2 15 well 12.5% Gels
with induced and uninduced
cultures.

- Spun @ 500 µl Culture 2 min
Aspirate off supernatant -
Resuspend pellet in 100 µl H_2O

10/5/95

Add 100µl 2X Dissociation Buffer
Mix well
Heat Samples 100°C 5min
Spin 45 min
Load 12.5µl onto gels.

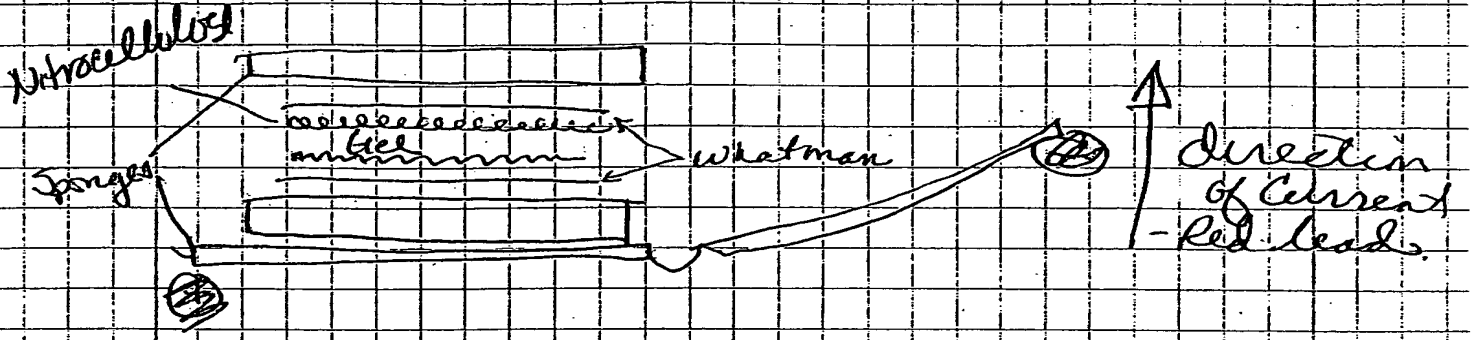
lane	Sample
1	Uninduced > M15 cells
2	Induced
3	" > HTPBY11 + PQE60 3-4
4	"
5	" > HMSAFZZ + PQE #19
6	"
7	" > HTPAN08 51bpATG + PD10 D9
8	"
9	Rainbow Marker
10	" > HTPAN08 51bpATG + PD10 D7
11	"
12	" > " " " + " C7
13	"
14	" > " " " + " A2
15	"

Run Gel - 150V 1hr
10% Gel - Set up Wet transfer.

Transfer Buffer:
25mM Tris pH 8.3
192mM Glycine
20% Methanol
Cold.

Set up Gel Sandwich in Support.

10/5/95

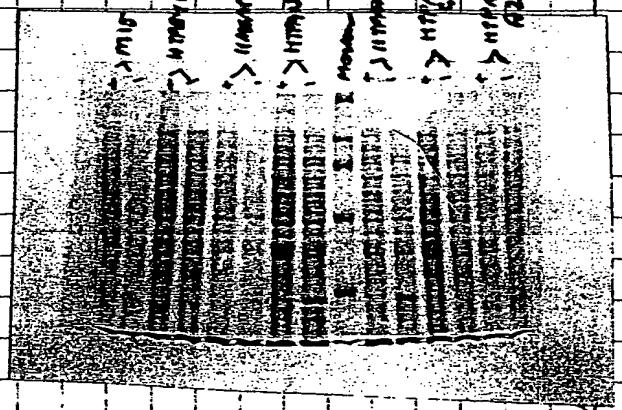


Run 100 V 1 hour.

Block Blot in
Western Blocking Buffer.
3% BSA (V)
0.02% Na - Azide
in PBS
Incubate at RT w/ Shaking
2 hrs
Pour off Blocking Buffer
Add 1° Ab in Western Blocking
Buffer at 1:100 dilution
Use Rabbit Antibody
#11941 - large Bleed
Incubate at Room Temp Overnight
with Shaking

With other gel
Stain
DESTAIN.

Take picture
of Gel



Inoculate 50 ml
LB+Amp Kan with
HTPA108 #9

10/5/95

HE2PM21 +PQE60-
Transform into M15 sup 4 cell
Thaw Chemically competent cells on ice
To 100 µl of thawed cells add 10 µl of Ligation
Incubate on ice for 1 hour
Heat Shock 42°C for 1 min
Place on ice
Add 400 µl LB
Incubate 37°C 1 hour
Plate onto LB-Amp/Kan plates
Incubate 37°C overnight

Transfections
H7PAN08 / H745B02
Remove Complete DMEM
Wash Cells 1X PBS 5 min
Remove Wash
Fix Cells 3% para formaldehyde in PBS
50 ml:
1.5 g Paraformaldehyde in 45 ml 1X PBS
Add 12.5 µl 10N NaOH
Heat 40-50°C to dissolve
Cool to Room Temp
fix Cells in 2 ml 3% para formaldehyde for 30 min at RT w/ shaking
Remove fixer
Wash Cells 10mM Glycine 1XPBS
5 min at RT w/ shaking

10/5/95

Remove Wash
To 1 set - add 1% Triton X100 in
1XPBS - into well.
Incubate 5min at RT.
To other set wash with 10mM Glycine
+ 1XPBS
Remove Triton X100 solution
Wash Cells - 10mM Glycine + 1XPBS - 5min
Remove Buffers
Wash cells 10mM Glycine + 1XPBS
5min w/Shaking
Remove Wash
Incubate Cells 25mM Glycine
+ 1XPBS at Room temp 1/2 hrs.
with Shaking

Block Cells in Antibody Binding
Buffer + 5% Goat Serum
20mM $Na_2H_2PO_4$ pH 7.0
0.5M NaCl
0.05% Tween 20
1% BSA
Incubate at Room Temp 2 hrs
with Shaking
Remove Blocking Buffer
Add 1° Antibody into Antibody
Binding Buffer
use HA - Tag purified Ab
1:1000 dilution
Incubate 4°C O/N with
Shaking.

10/6/95

Transfections - HT4SB02 / HTPAN03

Remove primary Antibody

10/6/95

Wash Cells 2X in 1XPBS + 10mM Glycine for 5min at Room Temp
Remove Wash
Add 2° Ab - at 1:2000 Dilution
RITC Conjugated Ab
in Antibody binding buffer + 2% Goat serum
Incubate at RT w/ Shaking for 2 hours Covered with Aluminum foil
Remove 2° Ab
Rinse Cells 2X in 1XPBS + 10mM Glycine at Room temp 5min
Remove all of Liquid
Add 1 large Drop of photon Mount
Cover with coverslip
Store 4°C Covered w/ Aluminum foil

HTPAN08 Western

Pour off 1° Ab
Rinse off filters in 1XPBS
Wash filters in 1XPBS 5min at R.T.
Add 2° Ab - Anti Rabbit Peroxidase - Dilute 1:2000 in 1XPBS
Incubate at Room temp with Shaking 2 hours
Rinse filters 1XPBS.
Wash filters 1XPBS - 5min at RT
Rinse filters w/ 50mM NaH_2PO_4 pH 7
Wash filters
50mM NaH_2PO_4 pH 7 for 5min at RT w/ shaking

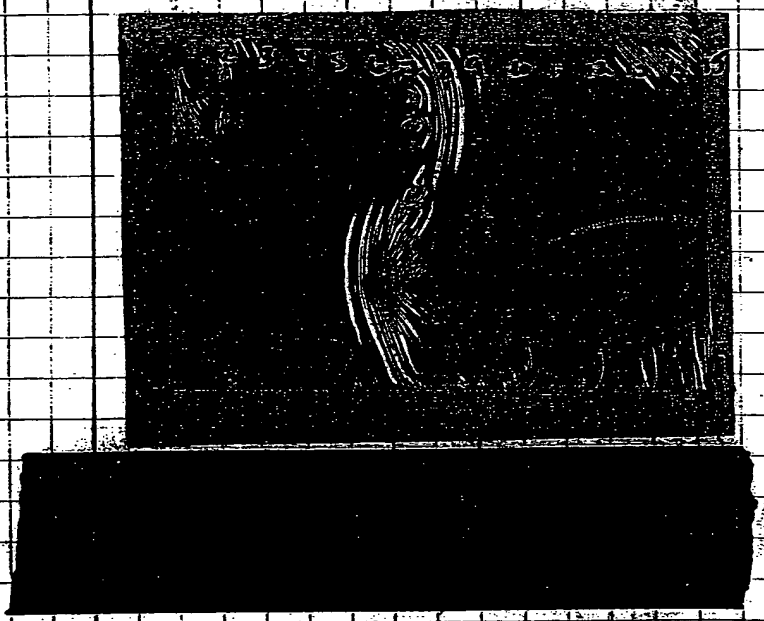
10/6/95

HTPAN08 Western

Add Substrate:

12.5 ml	50 mM NaH_2PO_4 pH 7
25 mg	B-NADH
25 ml	phenol
8.5 ml	H_2O_2
375 ml	NBT = Nitro blue Tetrazolium (10 mg/ml)

Incubate at RT until color change is seen and developed to desired darkness.
Stop Reaction with distilled H_2O.
Dry on filter paper



1 +] m15
2 −
3 +] HTPBH11
4 −
5 +] HMSAF22
6 −
7 +] HTPAN08 D9
8 −
9 Rainbow Marker
10 +] HTPAN08 D5
11 −
12 +] HTPAN08 C7
13 −
14 +] HTPAN08 A2
15 −

HTPAN08+PD10 #9 D9
Induction

Inoculate 1000 ml LB+Amp/Kan with 20 ml of overnight Culture

10/6/95

HTPAN08 PD10 D9 - Induction
Incubate 37°C w/aeration
till OD_{600} = 0.4 - 0.6 - Incubated
2 1/2 hours
Add 100mM IPTG to 2mM -
630ml → 12.6ml
Incubate 4 hours w/aeration
at 37°C
Spin Cultures 5K 30 min
Pour off Supernatant
Resuspend pellet a total of
70ml 6M GnHCl pH 8
Store 4C over the weekend

HE2PM21 +PQE60
Incubate 200ul LB+Amp/Kan
in 96 well dish.
Inoculate with Colonies -
72 of HE2PM21+PQE60 and
6 of PQE60 Bam/Nco
Incubate ~~37°~~ at Room Temp
over the weekend

10/9/95
HE2PM21 +PQE60 PCR.

Check Cultures for Inserts

10/9/95

HTPA108 pD10 D9 Induction

Spin Cells in 6M Gn HCl 8K
30 min
Transfer Supernatant to fresh tube
Store on ice

Prepare Ni-NTA Column
To Column add 5ml Resin Slurry
Wash with 30ml diH_2O
Charge Column by adding 30ml 0.10M $NiSO_4$
Wash with 30ml diH_2O
Equilibrate 30ml 6M Gn HCl pH8
Apply Supernatant
Allow to flow - Collect all flow through
Wash 50ml 6M Gn HCl pH8
- collect pH8
Wash 50ml 6M Gn HCl pH6.
- collect pH6
Elute Protein - 8ml 6M Gn HCl pH5
- Collect pH5
Strip Column - 50ml 6M Gn HCl pH2
- collect pH2
Store at 4°C till tomorrow -
Run on Gel

10/10/95

HERPM21 + PAE Co Inductions

Heat Samples 100C 5min
Spin 2 min
Load 12.5ul per well

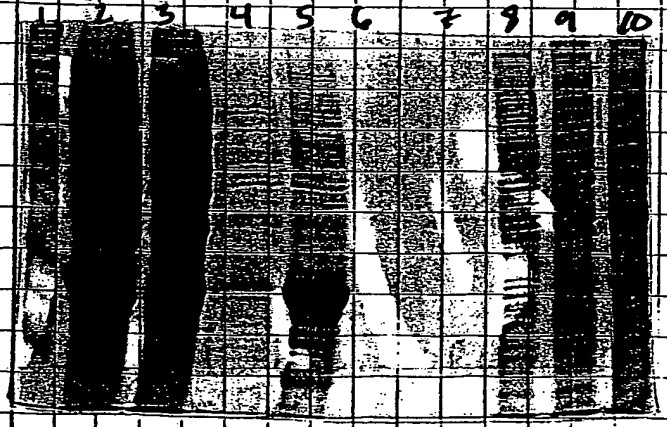
10/10/95

HTPAN08 PD10 D9.

Run samples on gel

H_2O 4.50 ul
Samples 50 ul
0.15 % NaDoC 75 ul
50 % TCA 50 ul

Mix well
Spin 10 min
Remove Supernatant
Resuspend pellet in 10 ul 0.2N NaOH
Add 10 ul 2X dissociation Buffer
Mix well
Heat 100°C 5 min
Run 20 ul on 12.5 % SDS-PAGE
Stacking gel
Run 150 V 1 hour.
STAIN
DESTAIN
Dry on Bio Rad Air Gel Dryer



1- Marker
2- Total Crude Extract
3- Flow
4 pH8
5- pH6
6- pH5
7- pH2
HTPAN08 PD10 D9
8- UN
9- 54
10- 55
HELPMP1 PDC00

10/10/95

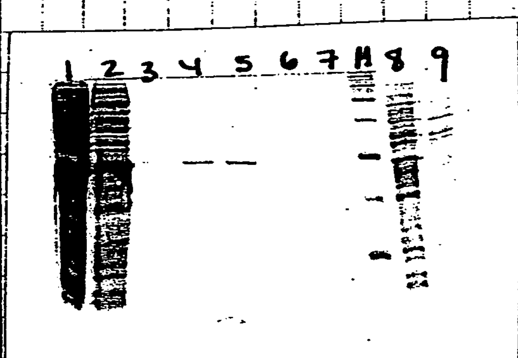
Looks like there is still a lot of protein ~~xxx~~ left in the flow-
Reapply to column
- pH 2 as off - Most of Protein came off of column

10/11/95

Regenerate Column.
30 ml H_2O
30 ml 0.1M $NiSO_4$
30 ml H_2O
30 ml 6M GmHCl pH8.

Add pH 6 to Flow from 10/10/95
Add 6M GnHCl pH 8
elute protein off of column - See pg 42 10/9/95

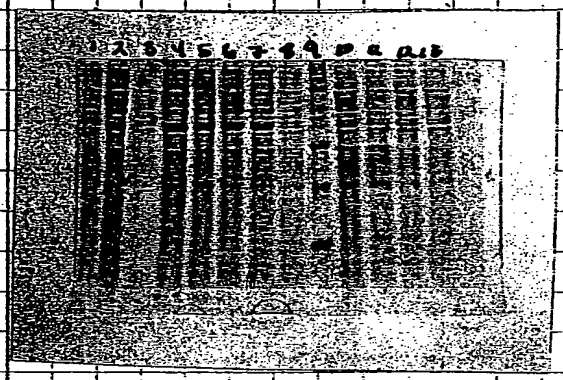
Run 50 ul on gel with 1Kb ladder



1 Crude Extract
2 - Flow
3 - pH8
4 - pH6
5 - pH5
6 - pH2
7 - pH5 from 10/9/95
M - Rainbow Marker
8, 9 > ~~HEEN~~ HEPAN08 + PD10 D9, ~~D8~~ D5

10/11/95

Run Remaining Samples of HERM21 HPEC60 on gel with Rainbow Marker
Stain Destain



1 HERM21 HPEC60 56
2 57
3 58
4 59
5 60
6 61
7 62
8 UV induced
9 Rainbow Marker
10 D9
11 D5
12 C7
13 A2

HERAN98 from 10/5/95

Inoculate LBt + 15mM MgCl2 with M15 rep4 & XL-1 Blue.

10/12/95

Make Chemically Competent XL-1 Blue and M15 rep4

To 2 - 2l flasks with 300 ml LBt + 15mM MgCl2
Add 3.5 ml of O/N culture
Incubate 37°C w/ Aeration 2 - 2½ Hours Until OD600 = 0.4 to 0.6.
Transfer Cultures to Sterile Centrifuge Bottles. Cool on ice 30 min.
Spin 5K for 30 min at 4C
Decant Supernatant
Resuspend pellets in Solution A ice cold. for every 600 ml resuspend in 300 ml

10/12/95

Solution A

50 mM $MnCl_2 \cdot 4H_2O$
50 mM $CaCl_2$
10 mM MES pH 6.3
- make Day ahead - store 4°C.

Let Cell suspension sit in ice
20 min
Spin Cells 5K 30 min
Resuspend cells in ice cold Solution A + 15% Glycerol
Resuspend in 3 ml for every 50 ml Starting volume - ~~N84ml~~
Aliquot 500 ul into Sterile tubes.
Quick Freeze on dry ice.
Store at -70°C

Reapply HIPAM08 SIbpATL PD10 D9 to Column.
Add More resin to Column Bed.
Regenerate Column
- See protocol pg 42 (10/9/95)

Run 50 ul on gel.

50 ul Sample
75 ul 50% TCA
50 ul 0.15% NaDOC
450 ul H_2O

Mix well Spin 10 min
Resuspend pellet 10 ul 0.2N NaOH
Add 10 ul 2X dissociation Buffer
Heat 100°C 5 min

10/12/95

Quick Spin 6 min
Run on 15% Acrylamide
Stacking gel 100V 1½ hrs
Stain 30 min 54°C
Destain O/N at RT.

F

HELP21 + PQE60

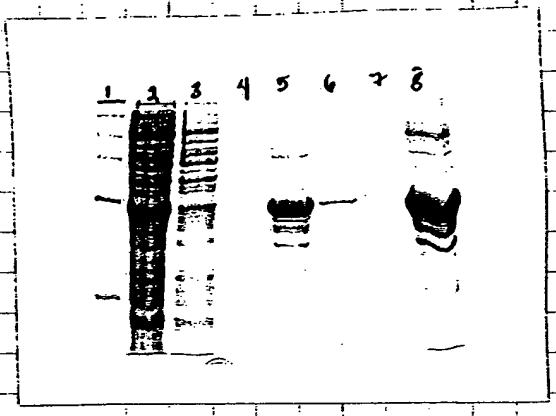
B. Mini preps
Spin 1.5 ml Culture 2 min
Remove Supernatant
Resuspend Pellet in 600 µl
STET + RNase & Lepozyme
Boil 5 min
Spin 10 min
Remove Pellet
Add 600 µl 13% PEG / 1.6 M NaCl
Mix well
Spin 10 min
Remove supernatant
Wash pellet 1000 µl 70% Ethanol
Spin 5 min
Remove Supernatant
Allow Pellet to air Dry
Resuspend pellet 50 µl TE
Run 1 µl on gel with 1 Kb ladder

Looks like DD DNA

May need to re-do

10/13/95

HTPANOX 5lbp ATG+ PD10 D1.



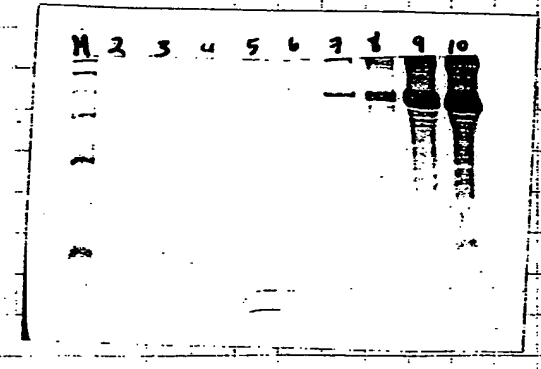
1 Rainbow Marker
2 Crude Extract
3 flow
4 pH 8
5 pH 6
6 pH 5
7 pH 3
8 pH 5 - from 10/11/95

10/16/95

Run Gel —
Quantitative gel of BSA. 10mg/ml.

2 10ng
3 50ng
4 100ng
5 500ng
6 1µg
7 5µg
8 10µg
9 100µg
10 600µg

Run on 15% PAA Stacking gel with Rainbow marker
150 V 1½ hrs.
STAIN
DESTAIN



10/10/95

Run Preparative Gel of HTPA08 61bp ATH + PB10 D9 pH6.

200 µl pH6.
150 µl 60% TCA
100 µl 0.15% N. DOC
800 µl H_2O.

2X

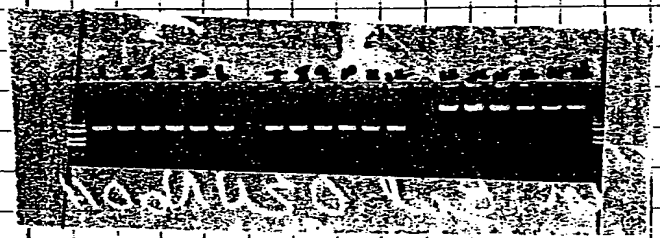
Mix well
Spin 10 min
Remove Supernatant
Resuspend Pellet in 400 µl 0.2N NaOH
Add 400 µl 2X dissociation Buffer
Run on 6% Preparative Stacking gel
100V 1½ hrs
Cut off part of Gel w/ markers and Stain
Save part of Gel
STAIN
DESTAIN.

Cut out band at appropriate size

Store Gel strips at 4°C in 15ml Conical Tube

10/25/95

Incubate 37°C 4 hrs
Run 1 µl on gel with 1Kb ladder



1	BamHI / BglII	pQE60
2	BamHI / NcoI	
3	BglII / BamHI	
4	BglII / NcoI	
5	NcoI / BamHI	
6	NcoI / BglII	
7	BamHI / BglII	pQE7
8	BamHI / SphI	
9	BglII / BamHI	
10	BglII / SphI	
11	SphI / BamHI	
12	SphI / BglII	
13	~~AspHI / BamHI~~ BamHI/Asp	pA2
14	BamHI / BglII	
15	BamHI / KpnI	
16	AspHI / BamHI	
17	BglII / BamHI	
18	KpnI / BamHI	

Add 150 µl TE
Add 20 µl 3M NaAcet.
Add 450 µl 100% Ethanol

Store -20°C

Run Gel of HTPAN08 Ecoli purified proteins to determine Concentrations

Samples: HTPAN08 185bp ATG 2/15
51bp ATG 2nd Lumid Elut 5/20
51bp ATG pH5 10/11
51bp ATG pH5 10/12
51bp ATG pH6 10/12

Use BSA - 10µg/µl as standard.

10/25/95

Run 10 ul of HTP AD Sample

500 ul H_2O
50 ul 0.15% NaDOC
75 ul 50% TCA
10 ul Sample

Mix well
Aspirate off Supernatant
Resuspend pellet in 8ul 0.2N NaOH
Add 8ul 2X dissociation Buffer
Heat 100°C 5 min
Run entire amount on 10% Acrylamide gel.

~~BA~~
BSA - 500 ng
750 ng
1 ug
2 ug
3 ug
4 ug
5 ug
10 ug
100 ug

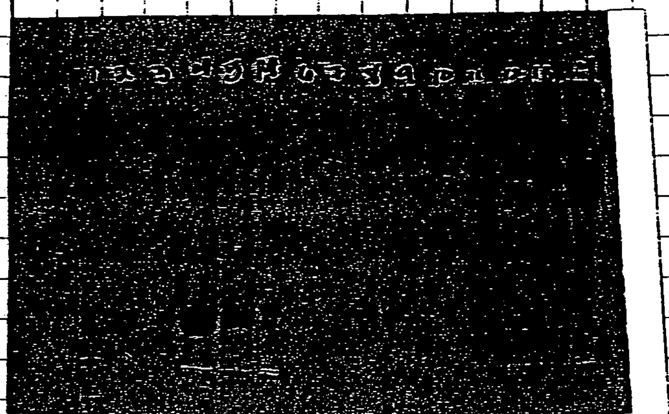
To all samples dilute to desired Amt. Add 10 ul 2x dissociation Buffer
Heat 100°C 5 min
Run entire Amt on gel with marker

Run 150 V 1.3 hrs
Stain 37°C 1/2 hr.
DESTAIN over night at RT

Send 100 ug Proteins to Rick King at Georgetown
2 Batches of ~~the~~ TGF α

10/26/95

Take Picture of HTPAN08 Gel



1 185bp 2/15 }
2 51bp 2nd elut } HTPAN08
3 51bp pH5 10/11 }
4 51bp pH5 10/12 }
5 51bp pH6 10/12 }

6 500 ng }
7 750 ng }
8 1 ug }
9 2 ug } BSA
10 3 ug }
11 4 ug }
12 5 ug }
13 10 ug }
14. 100 ug

HTPAN08	185bp 2nd		600	→	60ng/ul
HTPAN08	51bp 2nd		~~x~~	→	X
	51bp	10/11	300	→	30ng/ul
	51bp	pH5 10/12	10ug	→	1 ug/ul
	51bp	pH6 10/12	4ug	→	400 ng/ul

Set-up Samples for westerns -
Use:

				100 ng
(1) HTPAN08	185bp	60ng/ul	=	1.7 ul
(2)	pH5 10/12	1 ug/ul	=	0.1 ul
(3)	pH6 10/12	0.4 ug/ul	=	0.25ul.

(4) → Galk uninduced Cells

Run 3 Gels - Marker 1,2,3,4, Marker 1,2,3,4, Marker 1,2,3,4
will get a total of 9 blots.

10/26/95

Do Diffent Amounts of Samples

	50ng	200ng	500ng
185bp	0.83	3.33	8.33
pH5 10/12	0.05	0.2	0.5
pH6 10/12	0.125	0.5	1.25

for all Samples:

500 µl H2O
50 µl 0.15% NaDOC
75 µl 50% TCA

Mix well
Spin 10 min
Remove Supernatant.
Resuspend different Quantities of Protein in 10 µl 0.2N NaOH
Resuspend the 100 ng Samples in 4 µl 0.2N NaOH
Add equal Volume 2X dissociation Buffer

Run 10 µl of 100 ng Samples per lane and all of different Quantities

Marker	185bp 50ng	200ng	500ng	pH5 10/12 50	200	500	pH6 10/12 50	200	500	Marker

Run at 100 V 1.3 hrs.
Transfer Gels to Nitrocellulose
Western transfer - See pg 34

10/5/95

10/26/95

After transfer is complete.
Mark Markers.
Cut into Strip blots.
Store at 4°C in Western Block
Buffer: 3% BSA Fraction V
0.02% Na Azide in PBS.

Get Rabbit Ab from Youngsoo Kim

Rabbits 11940 + 11941
Large Bleeds 9/22/95
10/20/95.

Do Western.

Dilute Ab 1:200 in Western Block Buffer.

(1)	11940	9/22/95
(2)	11940	10/20/95
(3)	11941	9/22/95
(4)	11941	10/20/95.

Incubate at RT w/ Shaking overnight.

"PAPER Chase"

10/27/95

Western -

Pour off 1° Ab.
Rinse filters in 1X PBS.
Wash filters in 1X PBS
5 min at RT

10/27/95

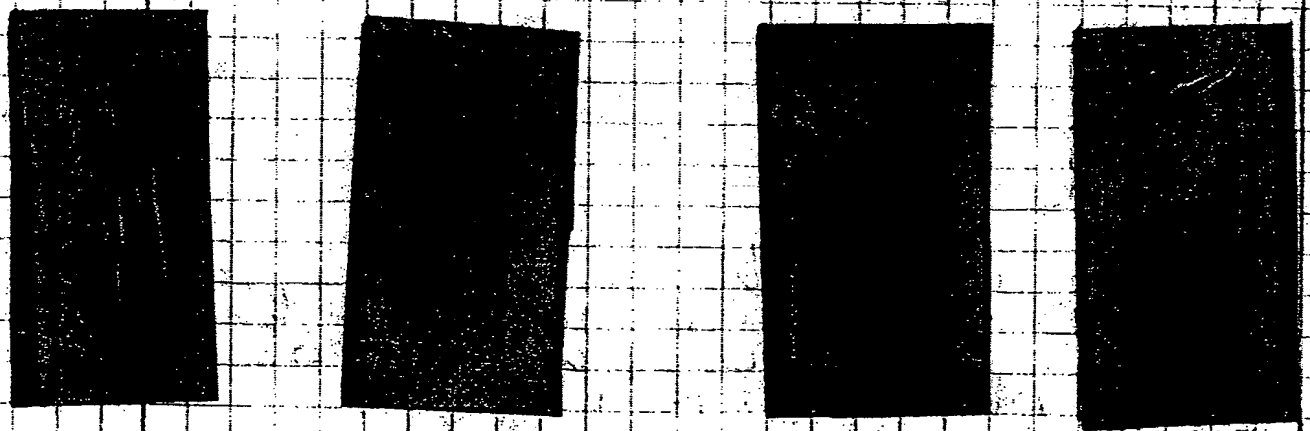
Add 2° Ab. @ 1 x PBS
1:1000 of Anti Rabbit Peroxidase Conjugate.

Incubate at RT w/shaking for 3 hrs
Pour off 2° Ab
Rinse filters 1 x PBS
Wash filters 1 x PBS 5 min at RT with Shaking
Pour off wash
Wash filters 50 mM NaH_2PO_4 pH7 at RT 5 min w/Shaking.

Add Substrate:

12.5 ml	50 mM NaH_2PO_4 pH7
25 mg	4-CN
25 µl	Phenol
8.5 µl	H_2O_2
375 µl	NBT

Mix well
Add to filters
Incubate till desired Color develops
Stop Rxn with di H_2O
1-Marker 2-185bp 3-pH5 4-pH6 5-Galk



11490 9/22 | 11490 10/20 | 11491 9/22 | 11491 10/20

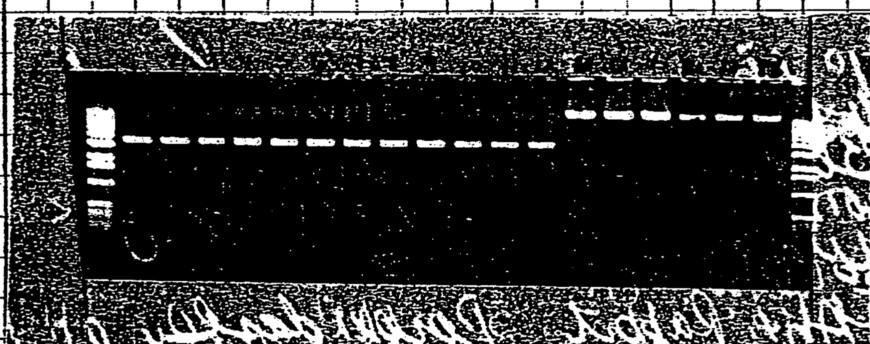
10/27/95

Set up mini gels for HTPAN 08.
follow same Guidelines as in PD 0302[illegible]. - 100ng of protein per well.

Store Blots in Western Block Buffer at 4°C.

PA2 + PQE 60/70 Vectors -

Spin 15 min
Remove Supernatant
Wash pellets 800ul 70% Ethanol
Spin 5 min
Remove Supernatant
Allow Pellet to Dry at RT for 30 min
Resuspend pellets in 50ul TE
Run 1ul on gel with 1 Kb ladder



1 Bam H1 / Bgl II
2 Bam H1 / Nco 1
3 Bgl II / Bam H1
4 Bgl II / Nco 1
5 Nco 1 / Bam H1
6 Nco 1 Bgl II

7 Bam H1 / Bgl II
8 Bam H1 / Sph 1
9 Bgl II / Bam H1
10 Bgl II / Sph 1
11 Sph 1 / Bam H1
12 Sph 1 / Bgl II

13 Asp 718 / Bam H1
14 Bgl II / Bam H1
15 Kpn 1 / Bam H1
16 Bam H1 / Asp 718
17 Bam H1 / Kpn I
18 Bam H1 / Bgl II

Store Vector #3 Box -20C

10/30/95

Dilute 1° Ab to see how far to dilute protein & Antibodies

Use Blots from 10/26 & 10/27

Blot			10 ml Western Block Buf
	1 →	1:200	60 µl
	2 →	1:300	33.3 µl
	3 →	1:400	25 µl
	4 →	1:500	20 µl
	5 →	1:750	13.3 µl
	6 →	1:1000	10 µl

use 1° Ab from #11490 10/20/95
Incubate O/N at Room temp with Shaking.

10/31/95

Remove 1° Ab
Wash Blots 1 X PBS
Wash Blots 3 x 5 min in 1 X PBS at Room temp w/ Shaking
Pour off Wash
Add 2° Ab
Goat-Anti Rabbit Peroxidase
1:1000 in PBS
Incubate at Room temp w/ Shaking
Pour off 2° Ab
Rinse Blots 1 X PBS
Wash Blots in 1 X PBS 5 min
at Room temp w/ Shaking

10/31/95

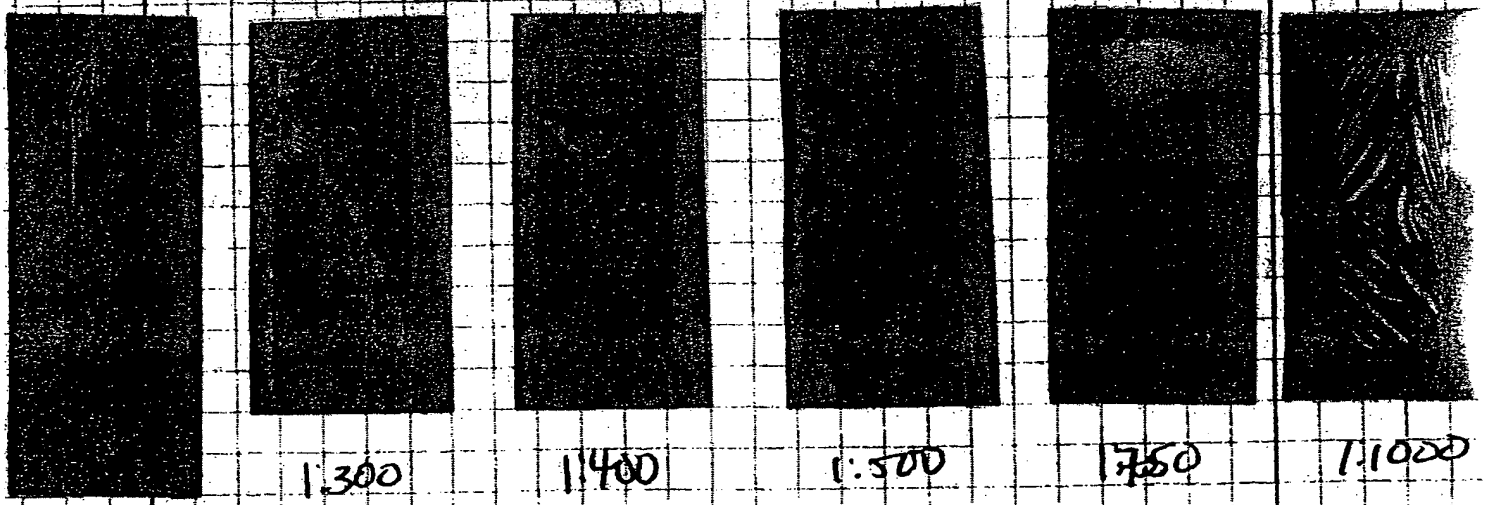
Pour off Wash
Washed filters 50mM NaH_2PO_4 pH7
at Room Temp for 5 min

Pour off wash.

Add Substrate.

125 ml	50mM Na_2HPO_4 pH 7
25 ml	phenol
25 mg	B-NADH
8.5 ml	H_2O_2
375 ml	NBT

Add to filters
Incubated to develop Color
Stop with the addition of
Qi H_2O



looks like even at 1:1000 dilution protein looks good

11/1/95

Add 1° Ab at 1:1000 to Western from 10/26/95 (pg 64)
Incubate overnight at room temp with Shaking
=
Will need to plate libraries to screen for full length gene of
HSXCB49 - interferon
Currently it is Henrik Olsen's project

11/2/95

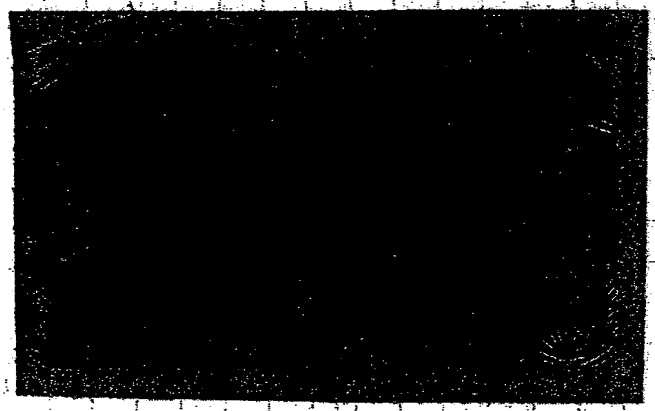
Remove 1° Ab
Wash Blots 1XPBS
Wash Blots 5 min 1XPBS at Room Temp w/ Shaking
Pour off Wash
Add 2° Ab at 1:1000 Dilution in 1XPBS - Anti Rabbit Peroxidase
Incubate at Room Temp w/ Shaking for 3 hrs
Pour off 2° Ab.
Rinse Off filters 1XPBS
Wash filters 1XPBS 5 min at RT with Shaking
Pour off Wash
Rinse filters in 50mM NaH_2PO_4 pH 7.0
Wash filters in 50mM NaH_2PO_4 pH 7.0 for 5 min at Room Temp with Shaking
Pour off Wash
Add Substrate

11/2/95

Substrate:

12.5 ml	80 mM NaH_2PO_4 pH 7.0
25 mg	B-NADH
25 µl	phenol
8.5 µl	H_2O_2 (30% Solution)
375 µl	NBT

Add Substrate to filters
Allow Color to develop to desired Darkness.
Stop Reaction with dH2O
Allow Filter to air Dry on Whatman paper



lanes:

1 - Marker		
2 - 50 ng	}	HTPAN08 1856bpATG
3 - 200 ng		
4 - 500 ng		
5 - 50 ng	}	HTPAN08 516bpATG pH 5 10/12
6 - 200 ng		
7 - 500 ng		
8 - 50 ng	}	HTPAN08 516bpATG pH6 10/12
9 - 200 ng		
10 - 500 ng		
11 - Marker		
12 - Galk Uninduced		

Titer HBJ library →

1:10 → 1:100 → 1:1000 $1:10^4$ $1:10^5$ $1:10^6$ $1:10^7$

plate out ~~1:100~~, $1:10^6$, $1:10^5$, $1:10^4$

11/2/95

Inoculate 200 ml LB + 10mM $MgSO_4$, 2mM Maltose with Frozen Stock of LE392
Incubate 37°C W/aeration Overnight

11/3/95

plate out HBJ, HSX, HFL
20 plates — 30,000 plaques/plate

HBJ — Titer: 60,000 → 6×10^5/ml
HSX — 2.4×10^9/ml
HFL — 2.2×10^{10}/ml

Span LE392 O/N Culture
BK. 15 min
Pour off Supernatant
Resuspend Cells in 10mM $MgSO_4$
OD600 = 2.0

To 1 ml Cells add phage

HBJ — 1 µl
HSX = 0.00025 µl
HFL = 0.000027 µl.

Incubate Cells + phage 37°C 15 min
Heat LB + 0.7% Agarose in Microwave to melt all of Agarose.
Aliquot 7 ml per tube and place in 50°C H2O Bath to Cool

After Incubation of Cells + Phage is complete

11/10/95

Computer Work.

11/10/95

11/13/95

Develop HSXCB49 1° Screens again.

Looks like there are a couple of ⊕ picks into 500µl SM Buffer

HSX - 1, 2, 3, 4
HFL - 1
HBJ - 1

PCR to test if insert is there.
Get FP03 primer from Laurie (LAI)

HSXCB49FP03 (3.2µM)	2	
M13R (3.2µM)	0.02	⊕ control plasmid DNA
10x dNTP	5	
10x PCR	5	
H_2O	35.8	
Taq	0.2	⊖ control H_2O
phage	2	
	50µl	

PCR - PROGRAM 58.

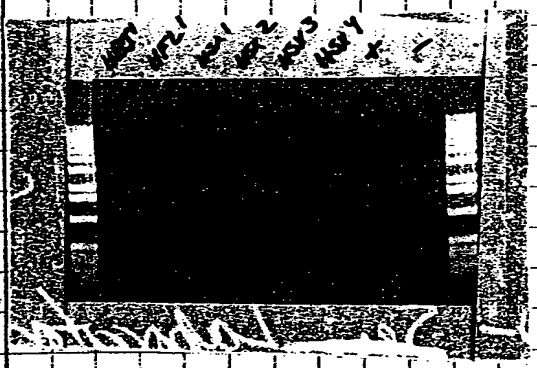
95°C	5min	
95°C	1min	30x
55°C	1min	
72°C	2min	
72°C	7 1/2 min	
4°C	Hold	

11/13/95

Plate 2° –
300 µl LE392 OD_{600} = 1
+ 2 µl of 2° phage

Incubate 37°C 15 min
Plate onto 150 mm NZY plates
in 7 ml NZY + 0.7% top agar.
Incubate 30°C O/N.

Run 10 µl of PCR Reaction on
1% TAE gel with 1 kb ladder



Nothing worked
even ⊕ control
Get another
primer from
Laurie & Try
again.

Received Primers –
Some –
Diluted to 3.2 pmol/µl for
Sequencing

Jian Ni → Requested Rabbit Antibody
Sera –
I gave him 1 ml each:
#11491 – 10/22/95
#11490 – 10/22/95

11/17/95

Picked 2° ⊕ clones of HSX-

~~No PCR ⊕'s~~

Only HSX 2° - 3 had any ⊕.

Picked 12 into 200µl SM Buffer

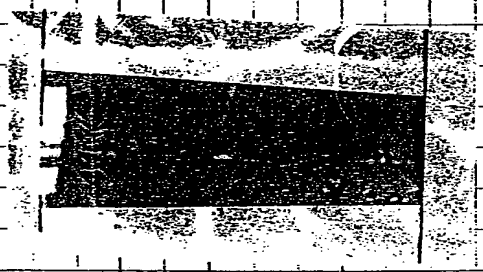
Do PCR.

M13R	0.05
M13F	0.05
10X	10
10X	10
H_2O	77.6
Taq	0.3
Phage	2
	100

PCR Prog 58.

95°C	5min	
95°C	1min	30X
55°C	1min	30X
72°C	2min	30X
72°C	7½min	
4°C	Hold	

Run 10µl on gel



Alan M Rubin
11/29/95

11/20/95

Analyzed sequence of Egf Clones - They all blast as part of the egf/fgf family.

Worked on HOUCQ17 Clone - See # 289

90

11/22 - 11/26 Out of office

11/27/95.

Carrie Received clones from Stephens

DATE 11-21-95

CLONE PROCESSING SHEET

Clone I.D. HOAA046, HELDQ06, HCEEX61, HOEDF83, HTEBY23 (transfer to Cat 11-20-95)

Requested By Steve Ruben

Transfer Folder 11-17-95

Processed By AMG

DATE 11.20.95

CLONE PROCESSING SHEET

Clone I.D. HTEGS33

Requested By STEVE RUBIN

Transfer Folder 11/20/95 TRANSFER

Processed By JJM BCI

Inoculate 250 ml TB+Amp with Culture

Carrie Sick so Inoculate HEPCU24 for her.

Incubate BTC O/N w/aeration

12/4/95

HNEDJ57

		62X
95-117	0.08	4.96
M13R	0.02	1.24
10X dNTP	3.2	198.4
10X PCR	3.2	198.4
H2O	22.3	1382.6
Taq	0.3	18.6
@ Phage	2	
	32	

12/6/95 ~~Should Be HSABU83!~~

Should be OK since 95-117a is for HSABV83 and 117b is for HNEDJ57

Rec'd Primers for HTPAN08504 51bp + PQE6

			X 12X
3' Hind III	#14388	2	24
5' Nco I	#2888	4	48
10X dNTP		3.2	38.4
10X PCR		3.2	38.4
H2O		16.3	195.6
Taq		0.3	3.6
DNA (cong/ul)		1	10
		32	32ul / Tube

Ran PCR Prog 58

95°C 5 Min

95°C 1 min ⎫
55°C 1 min ⎬ 30x
72°C 1 min ⎭

72°C 7½ min

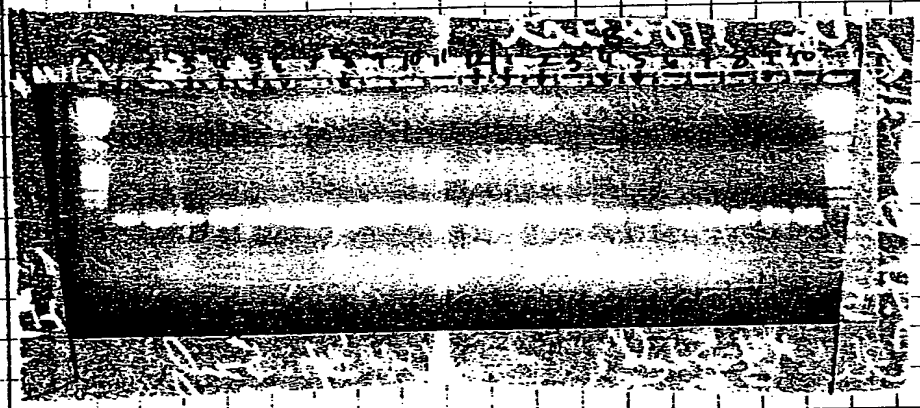
4°C Hold

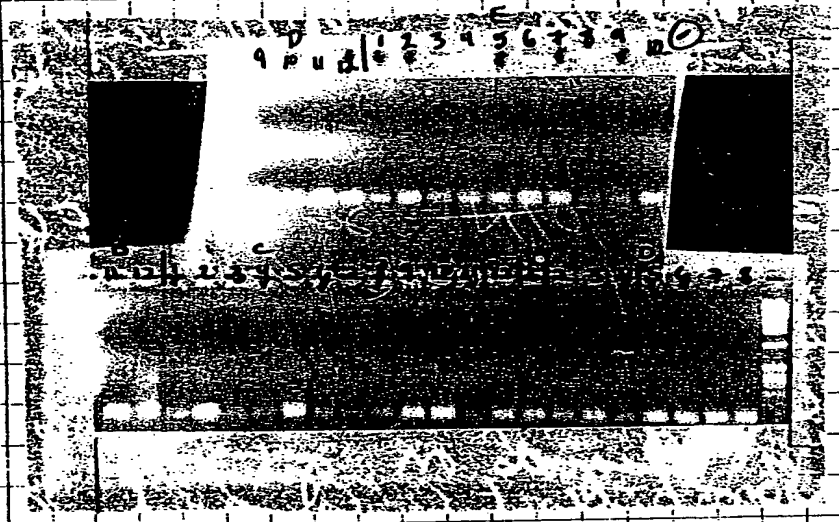
Ran 10ul on gel with 1Kb ladder

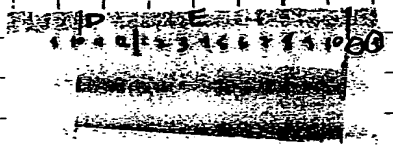
Ran 2ul of HTPAN08 PCR

[redacted]

12/4/95







Pick + for 2° Screenings

A-1	A8	B5	C8	D12	E9
A-3	A12	B9	C11	E1	
A-4	B1	C1	D4	E2	
A5	B2	C4	D5	E5	
A-6	B4	C6	D6	E7	

Dilute 1:1000 plate 10 ul onto 100mm NZY Plates

Incubate 30°C O/N

12/4/95

PEG PDT PCR Product
ethanol Wash
Resuspend pellet in 20ul TE

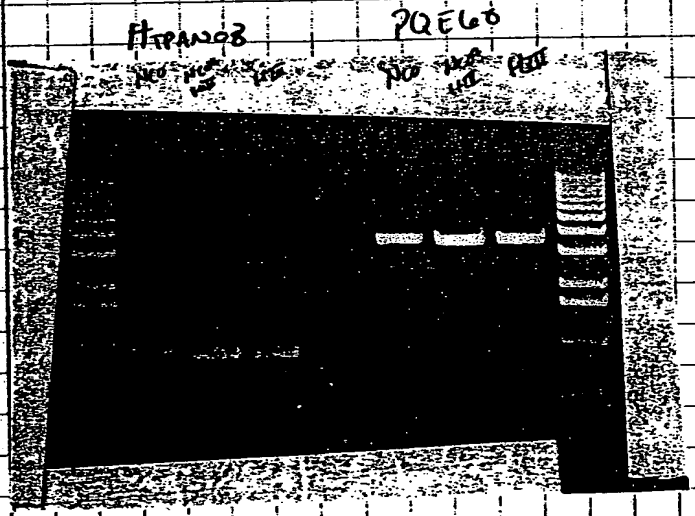
Set up Digestion:

	NCO	NCO/HIII	HIII
10X #4	#4-5ul	4 4-5ul	#2-5
H_2O	29ul	29ul	29
DNA	15ul PEG	15ul	15
Enzyme	1ul NCO	05/05	1 ul HIII
	50ul	50ul	50ul

Incubate 37°C O/N - Set up PQE60
Digests the same as above

12/5/95

Run 10ul of Digests on gel with
1Kb ladder



12/5/95

Add other enzyme
- HIII to NcoI Digests
- NcoI to HIII Digests
Incubate 37°C ~~[illegible]~~ 4hrs

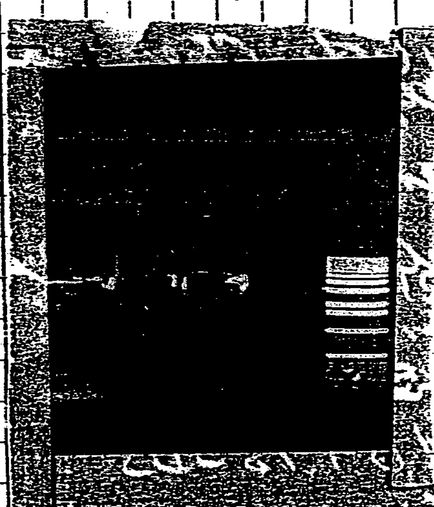
~~[illegible]~~
Run PQE60 on 0.8% ~~Agarose~~
LMP Agarose gel w/ 1kb ladder

PEG ppt HTPAN08S04 Products.
Resuspend in 20 µl TE.

Gel isolate PQE60 HIII/Nco Digests
will be PQE60.

Gene Clean.
gel slice

Resuspend & elute
in Total of 40 µl.



Set up ligations

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
HTPAN08 HIII/Nco	3	3	3							3						
HTPAN08 Nco/HIII				3	3	3					3					
HTPAN08 Nco & HIII							3	3	3			3				
PQE60 HIII/Nco	1			1			1						1			
PQE60 Nco/HIII		1			1			1						1		
PQE60 Nco & HIII			1			1			1						1	
10X Buffer	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
H_2O	13	13	13	13	13	13	13	13	13	14	14	14	16	16	16	17
T4 ligase	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

12/5/95

Incubate ligations at 16°C Overnight.

HNEDJ57 2° did not grow dense enough
to plate
at 1:500 plate 10ul.
Incubate 34°C. 5 hrs.

Most plates look good.
a couple look like they need to
be replated at a higher concentration
Incubate 30°C O/N.

12/6/95

Transform ligations into Chemically
competent DH5 up 4 cells

Thaw Chemically Competent MK5 cells
on ice
To 100 ul Competent cells add 10 ul
of ligation reaction
Let Sit on ice 1 hour
Heat 42°C for 1 min
Chill on ice
Add 400 ul LB
Incubate 37°C 1 hr
Plate 150 ul onto LB + Amp plate.
Incubate 37°C O/N.
④ Control pQE60 plasmid DNA

HNEDJ57 ~~HA~~ Shared by HSHBV83

2° Grew OK.
Chill plates at 4°C 1 hr

12/6/95

Do plate lifts w/ orientation marks.

Denature lifts 30 min
0.5M NaOH / 1.5M NaCl

Then Neutralize 5 min
0.5M Tris pH 8 / 1.0M NaCl

Allow filters to Dry at RT.

Prehybridize in 2x PIPES / 50% Formamide
overnight at 42°C.

12/7/95

Probe Human Tissue Northerns

HE20M21 → H. Fetal (F) & H. MTN II (M)
HTPAN08 → H. MTN I (H) + Cancer (C)

Prehybe in Express Hyb 42°C.
2 hrs.

Make Probe -
HE20M21 Xho/R1
HTPAN08 Sal Xho/R1
HSHBV83 Xho/R1

DNA	3
H_2O	21
5X Primer	10
	34

Heat 100°C 5 min
Quick Spin
Place on ice

12/7/95

Add
5X P^{32} dATP Buffer 10
^{32}P dATP 5
Klenow 1
50 µl

Incubate 37°C 8 min
Spin Through G-25 Column
Count 1 ul

SAM	POS	CH		CPM	
HE2PM21	1	135	1	787036.00	~70µl
HSHBV83	2	136	1	693160.00	~70µl
HTPAN08	3	137	1	674388.00	~70µl

Add 5ul Salmon Sperm DNA
to probe
Heat 100°C 5 min
Quick Chill
Quick Spin
Add probe to HE2PM21 Northerns
and HTPAN08 Northerns.
Incubate 42°C 4hrs
Wash Northerns
3X 65°C 0.2X SSC / 0.1% SDS
Put on film
-80°C Overnight

For HSHBV83-
Pour off prehyb from Filters
Add probe
Add denatured probe to hyb
Incubate 42°C O/N

12/7/95

HTPAN087 + PQE 6

Pick Colonies into LB + Amp, Kan
media in 96 well dish
Pick 12 each of 1-9
6 each of 10-15

Incubate 37°C w/ aeration 3 hrs
Set-up PCR.

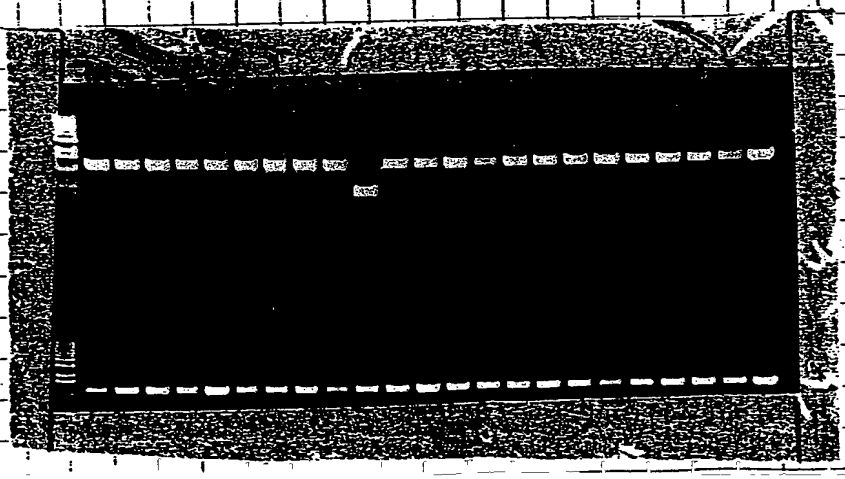
		x 150 x.
5' PQE	0.2	30
3' PQE	0.1	15
10x PCR	3.2	480
10x dNTP	3.2	480
H_2O	23.1	3465
Tag	0.2	30
Cult.	2	
	32	30 ul / well.

PCR Prog 66.

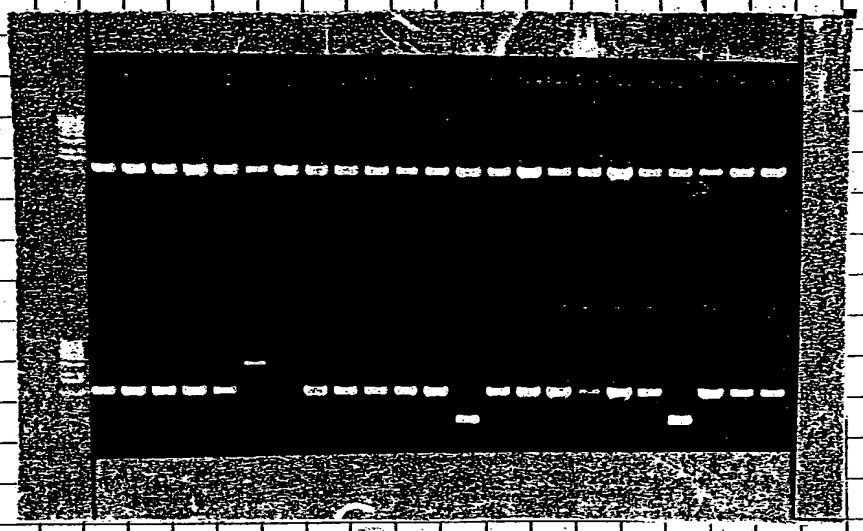
95°C	5min	
95°C	20sec	30x
55°C	20sec	
72°C	1min	
72°C	7 1/2 min	
4°C	Hold.	

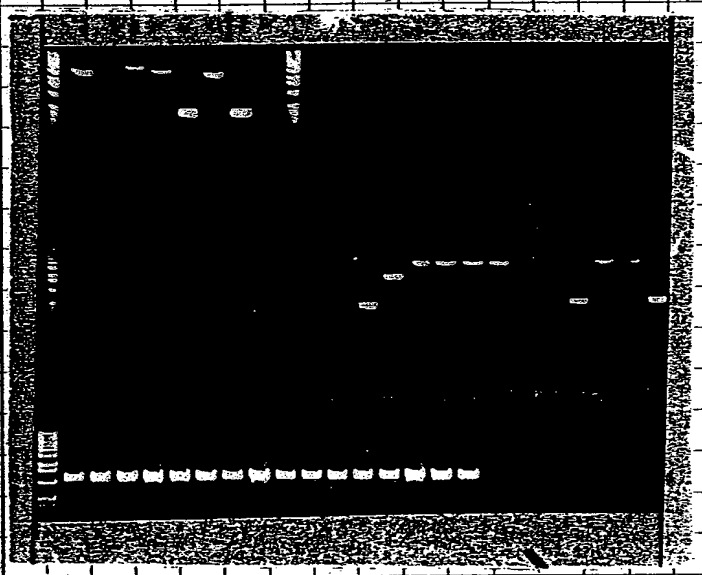
① Control PQE60
② Control NS DNA

Run 10 ul on gel w/ 1Kb ladder



12/7/95





Pick Clones for Boiling Minipreps

1-1	3-8	5-4	7-1
1-2	3-10	5-7	7-2
1-3	3-11	5-8	7-3
1-4	3-12	5-9	7-4
2-5	4-1	6-9	8-5
2-6	4-2	6-10	8-6
2-7	4-3	6-11	8-7
2-8	4-4	6-12	8-8
9-9	9-11	9-10	9-12

into 5ml TB+Amp/Kan

12/8/95

Boiling Mini preps.

Spin 2ml Culture 5 min

Remove Supernatant

12/8/95

Resuspend pellet in 800 µl STET
+ RNase & Lysozyme.
Heat 100°C. 5 min
Spin 15 min
Transfer 750 µl Supernatant to
fresh Tube.
Add 750 µl PEG / NaCl
Mix well
Spin 15 min.
Remove Supernatant
Wash pellet 1000 µl 70% Ethanol
Spin 5 min
Remove Supernatant.
Allow pellet to air dry.
Let go too long - 2 hrs
Pellet very hard to resuspend.
Add 20 µl TE to pellet
Store 4°C over weekend.

HSHBV83
Wash filters 0.2XSSC / 0.1% SDS
3X 65°C
Put filters on film.

Northern.
Developed Film.
Very faint
Re-expose -80°C over the Weekend.

4/11/95

HSHBV83 2°
Pick 2° plugs into 200 µl Sm Buffer

Screen PCR.

95-112	0.1	95-114	0.1
M13R	0.05	M13R	0.05
10XdNTP	5	10XdNTP	5
10XPCR	5	10XPCR	5
H_2O	34.6	H_2O	34.6
Taq	0.25	Taq	0.25
Phage	5	Phage	5
	50 µl		50 µl

PCR Program 58

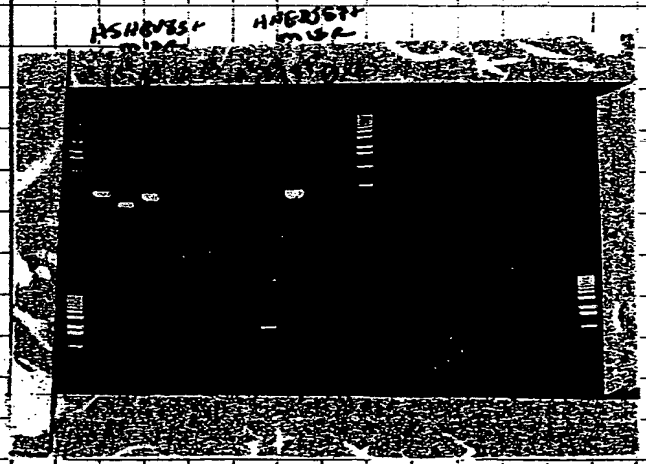
95°C	5min	
95°C	1min	30X
55°C	1min	30X
72°C	1min	30X
72°C	7 1/2min	
4°C	Hold	

Check Primer pairs on HSHBV83 Plasmid DNA HNEDJ57 Plasmid DNA

Use 91-87 which should be HNEDJ57 Specific

Run 1% gel with 1Kb ladder

13-1
13-2
13-3
15-1
15-2
15-3
17-1
20-1
20-2
20-3



Inoculate XL-1 MRF' SOLR LE392 to Excise

Resuspend H7PANO8 +PQE60 Clone 12/11/95
in 200ul TE
Run 2ul on gel w/ 1Kb ladder

Set up Digests.

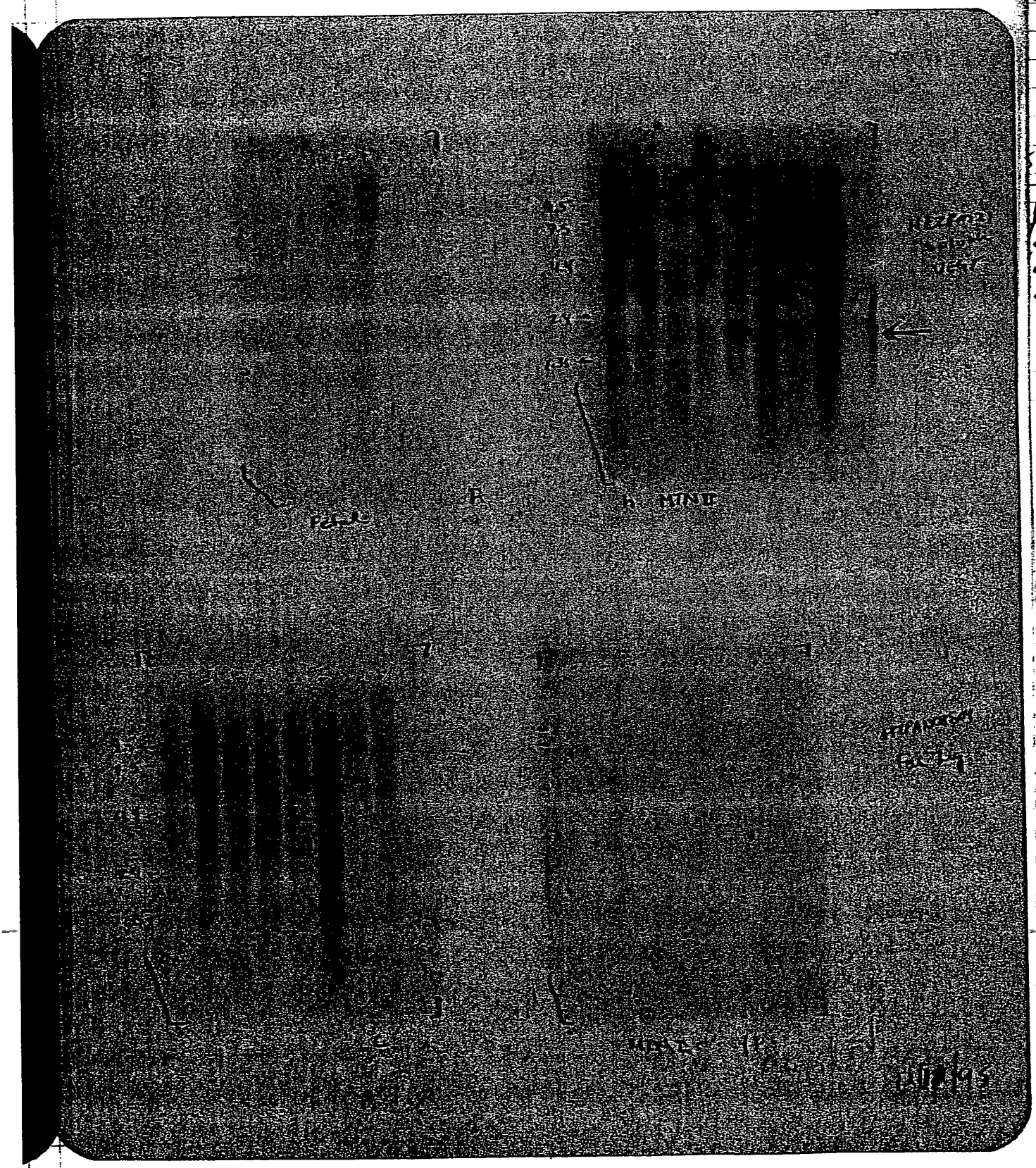
		38X
DNA	5	
10X#4	3	114
H2O	21.8	828.4
NcO	0.1	3.8
HIII	0.1	3.8
	30	

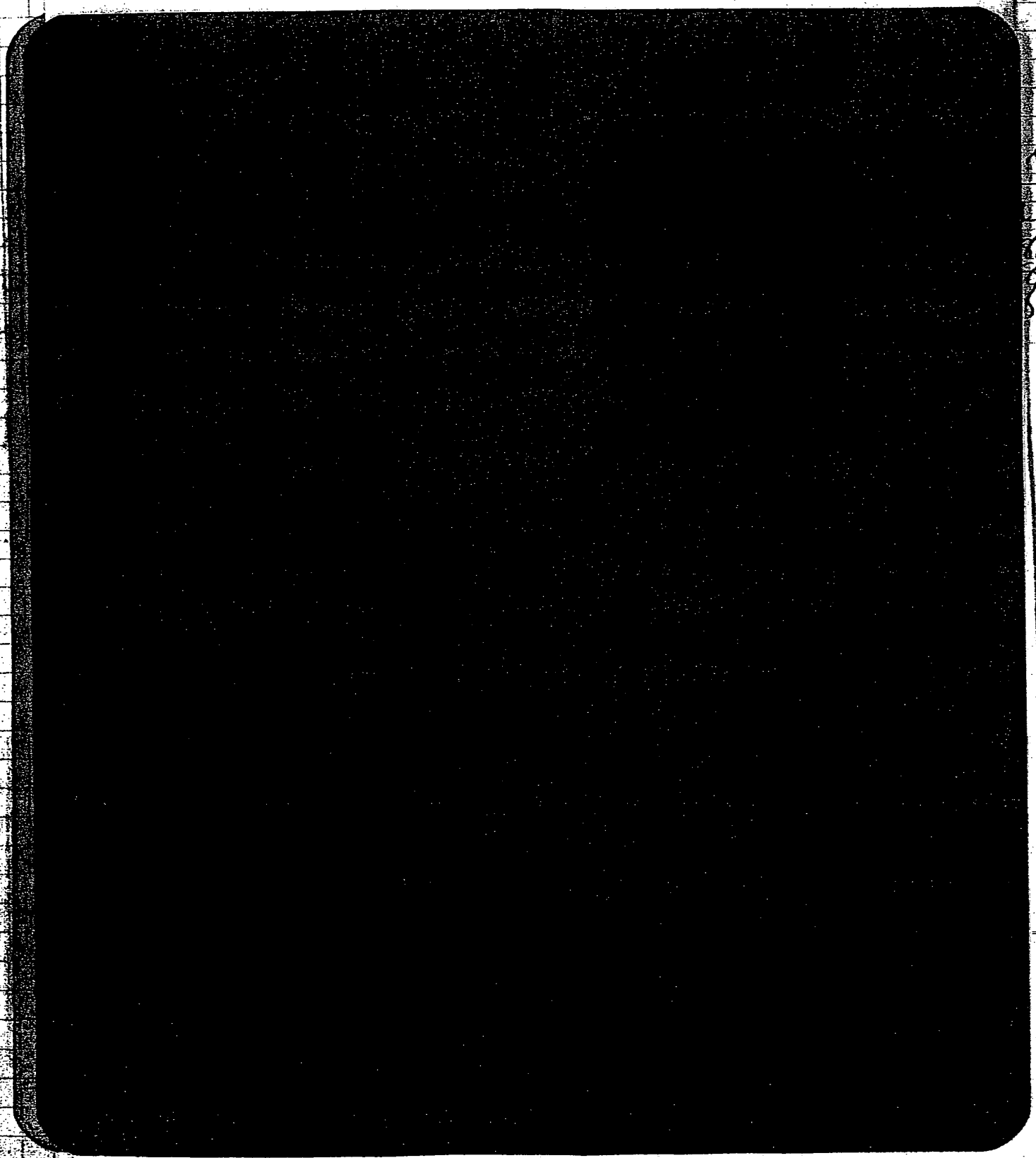
Incubate at Room Temp overnight

Small Scale induction:
Inoculate 500ul of LB+Amp/Kan with
50ul of O/N culture of H7PANO8+PQE60.

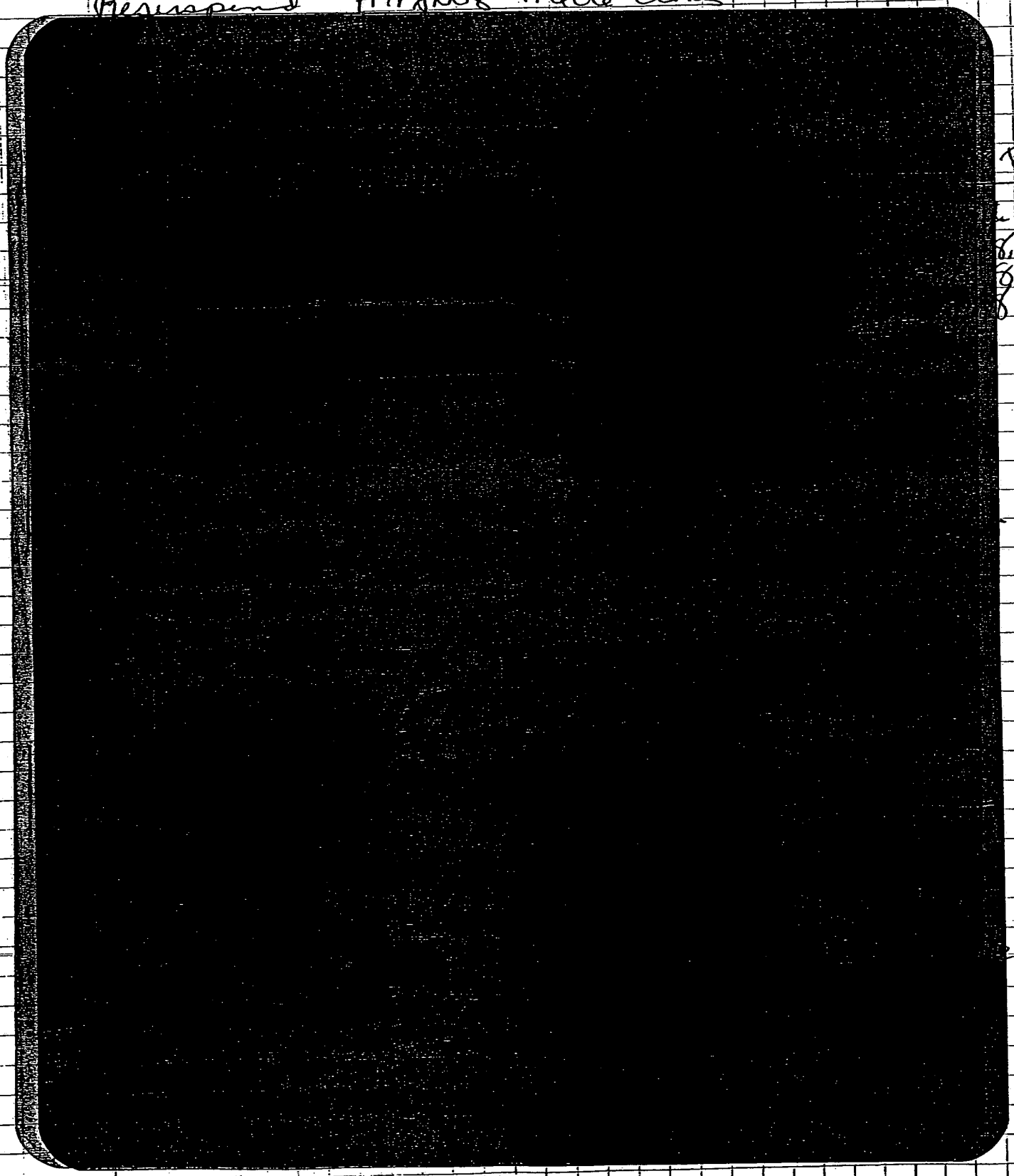
Incubate 37°C 1½ hrs
Add 11ul of IPTG (100mM) to 2mM.
Incubate 4hrs w/aeration 37°C.
Spin culture 5m
Resuspend pellet 40ul H_2O.
Add 40ul 2X dissociation Buffer
Store -20°C & Run on 15% Gel.

Develop Northern.
Difficult to say if there is anything
Strip blots 0.5% SDS in 250ml H_2O
Heat 5min in microwave
Place blots in 0.5% SDS.
Incubate 10 min.
Repeat until No counts can be heard


P
h HINDIII



Resuspend HTPANOR TPQEC6 Clone 12/11/95



12/12/95

Run 10 µl of Induced Proteins on 15% SDS-PAGE Gel.

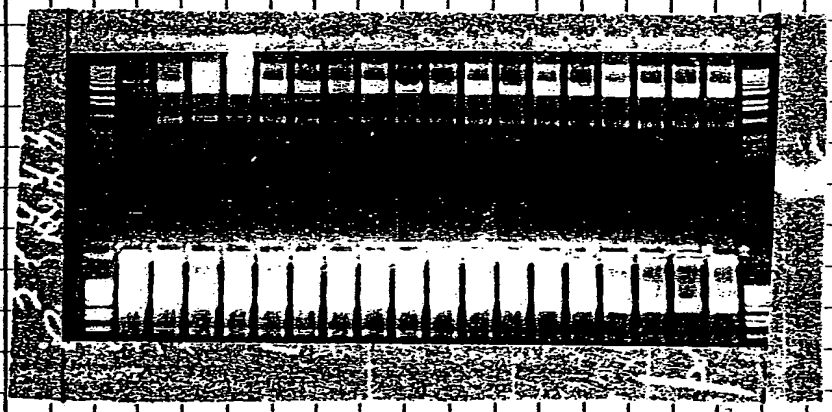
	Gel 1	Gel 2	Gel 3	Gel 4
ln 1	Marker	3-10	5-9	8-6
2	1-1	Marker	6-8	8-7
3	1-2	3-11	Marker	8-8
4	1-3	3-12	6-9	Marker
5	1-4	4-1	6-10	9-7
6	1-5	4-2	6-11	9-8
7	2-5	4-3	6-12	9-9
8	2-6	4-4	7-1	9-10
9	2-7	4-5	7-2	9-11
10	2-8	5-5	7-3	9-12
11	2-9	5-6	7-4	9-9 ON
12	3-8	5-7	7-5	2-5 ON
13	3-9	5-8	8-5	4-3 ON
14	2-5 ON	4-3 ON	6-12 ON	6-12 ON

Run 150 V 1½ hrs.
Stain 30 min at 57°C.
DeSTAIN 2 hr at 37°C
Dry Gels.

12/12/95

Can't tell Do Small Scale inductions

HTPAN08 + POE6.
Nco I + II digests

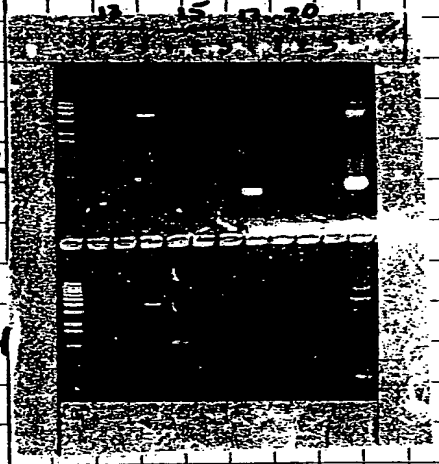


All clones look correct submit for Sequencing

Rescue HSHBV83 20-1
20ul phage + 100ul XL-1 Blue MRF' OD_{600}=1.0
+ 10ul Ex Assist
Incubate 37°C 15min
Add 2ml LB.
Incubate 37°C 2hrs.
Heat @80°C 15min
Spin - Plate ul 15ul/10ul + 200ul SOLR

12/15/95

Run 10 µl on gel w/ 1KB ladder



On Monday. Plate
13-3
15-1
20-2
17-1

HSHBV83

HTPAN08 + PQE60.

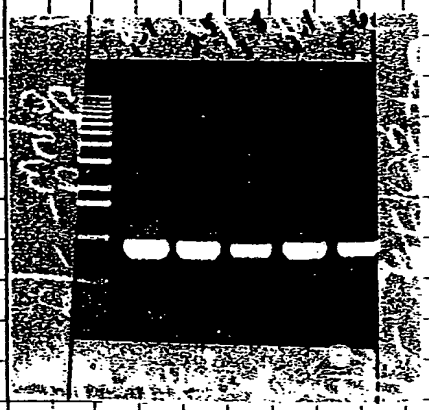
PCR 1-1, 2-5, 3-8, 4-1, 5-6
with T7 PQE60/70 and 3' HA1
8 tubes of 32 µl

		40X.
T7 PQE 60/70 (10 pmol/µl)	1	40
HTPAN08 3' HIII	2	80
10x dNTP	3.2	128
10x PCR	3.2	128
H2O	20.3	812
Taq	0.3	12
DNA (5ng/µl)	2	
	32	

PCR Program 66.

12/15/95

Run 5 µl on gel w/ 1Kb ladder



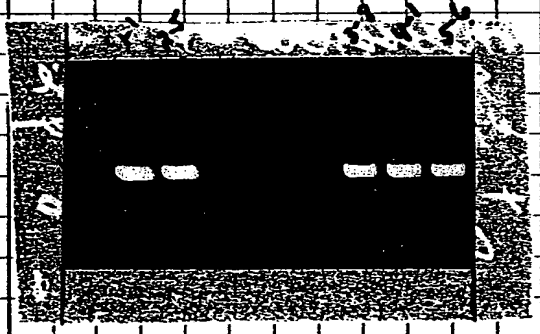
Combine the 8 tubes Ppt Reaction with Equal Volume PEG/NaCl

Wash pellet 1000 µl 70% Ethanol

Allow Pellet to Air Dry

Resuspend pellet in 40 µl TE

Run 1 µl on gel with 1Kb ladder



Submit 1-1 & 2-5 for TNT-T7

along with HESCT26 for TNT-T3

12/18/95

HSHBV83:

Excision & Rescue:

200 µl SOLR OD_{600} = 1.0

10 µl Rescue stock

Incubate 37°C 15 min

β 3' Delta

Develop [redacted] 12/21/95

Set [redacted]

		3						7	8	9
HSKBN0		3								
HMSA 3' Delta	[redacted]									
HM5A	[redacted]									
PGC	[redacted]							2		
GP PAZ DS	[redacted]								2	
10X T4 Buffer		2			2		2	2	2	2
T4 Ligase / Clonetech Petal		1	Clonetech / Human MTN II				1	1	1	1
Clonetech Human MTN I / Human MTN II H2O		12	12	12	14	14	14	15	15	17

Incubate 4°C over Break

12/22/95 1 Personal Day OFF

12/25/95 Christmas Day OFF

12/26 – 12/29/95 Company Break.

1/1/96 New Years Day OFF

1/2/96 1 Vac Day Off

1/3/96 – 1/5/96 3 Personal Days OFF

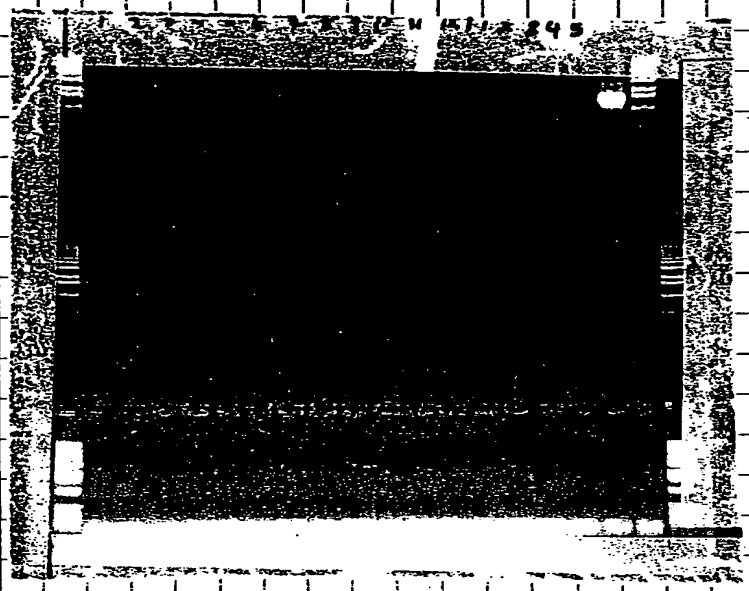
1/8/96 – 1/10/96 Company Closed Due to Weather.

1/11/95

Write in note book – Write progress report for Steve.

12/20/95

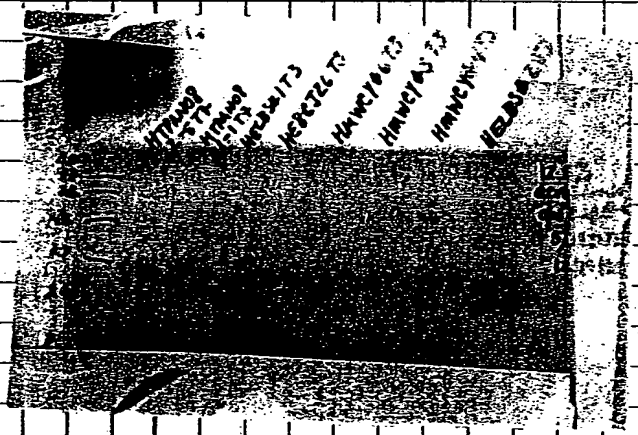
Run 10 µl on gel with 1Φ Kb ladder
1Φ USAF 22+PAR

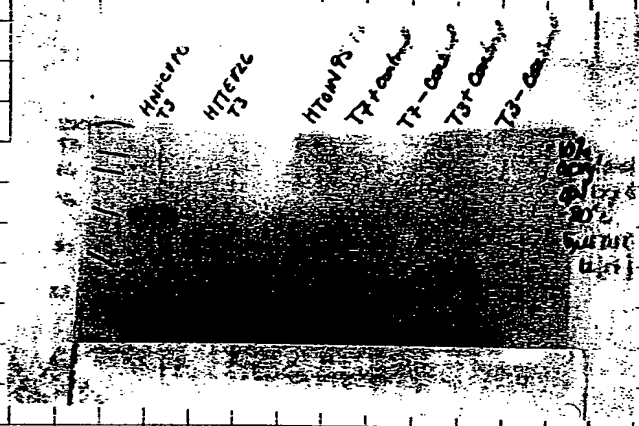


	TNT RESULTS 12/19/95		
INVESTIGATOR	SAMPLE NAME	EXPECTED PRODUCT SIZE KDa	OBSERVED PRODUCT SIZE KDa
ANN KIM	HTPAN08+PQE6 #1-1	33	33
ANN KIM	HTPAN08+PQE6 #2-5	33	33
ANN KIM	HE8CJ26	30	NO PRODUCT OBSERVED (background band at 30)
JING SHAN HU	HMWCY #6	30	NO PRODUCT OBSERVED (background band at 30)
JING SHAN HU	HMWCY #5	26	NO PRODUCT OBSERVED
JING SHAN HU	HMWCY #4	33	NO PRODUCT OBSERVED (background band at 30)
JING SHAN HU	HELBS #2	37	MAJOR BAND AT ~25 (others at 29 & 36)
JING SHAN HU	HELBS #1	30	MAJOR BAND AT ~25 (others at 29 & 36)
BRENT KREIDER	HNFCV70	92	TRIPLET BANDS AT 44,46 &48, MINOR BAND AT 30
BRENT KREIDER	HTTEV26	110	MAJOR BAND AT 36 (others at 30,40 &27)
LAURIE INSCORE	HTOIN95	48	NO PRODUCT OBSERVED
T7 POSITIVE CONTROL	DNASE 02-105	33	33 (gel loading problem, but band is visible)
T7 NEGATIVE CONTROL	NO TEMPLATE	NONE	NONE
T3 POSITIVE CONTROL	HCACI93	33	33
T3 NEGATIVE CONTROL	NO TEMPLATE	NONE	NONE

12/20/95 ℔

TNT Results Continued





12/20/95

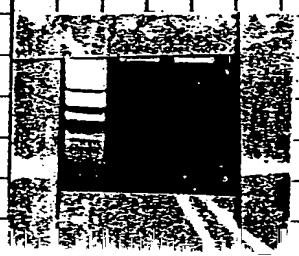
PPT Digests of

HMSAF2D 3' Delta Ass / 3' Bgl 11
HSKBN09 HTA / Sp6.T
Wash Equal Volume PEG / Wash
-20 C Overnight

12/21/95.

Run GPPA2 Digests on & Gene Cleaned fragments on gel.

Looks good.



Develop film + MNT 12/21/95

Set up Ligation:

	1	2	3	4	5	6	7	8	9
HSKBN09. HIII / SphI	3	—	—	3	—	—	—	—	—
HMSAF22 3'Delta Asp / BglII	—	3	—	—	3	—	—	—	—
HMSAF22 3'Asp / BglII	—	—	3	—	—	3	—	—	—
PQE 7 HIII / Sph	2	—	—	—	—	—	2	—	—
GP PA2 Asp / BglII	—	2	2	—	—	—	—	2	~~2~~
10X T4 Buffer	2	2	2	2	2	2	2	2	2
T4 Ligase	1	1	1	1	1	1	1	1	1
H2O	12	12	12	14	14	14	15	15	17

Incubate 4°C Over Break

12/22/95 1 Personal Day OFF

12/25/95 Christmas Day OFF

12/26 — 12/29/95 Company Break.

~~12~~ 1/1/96 New Years Day OFF

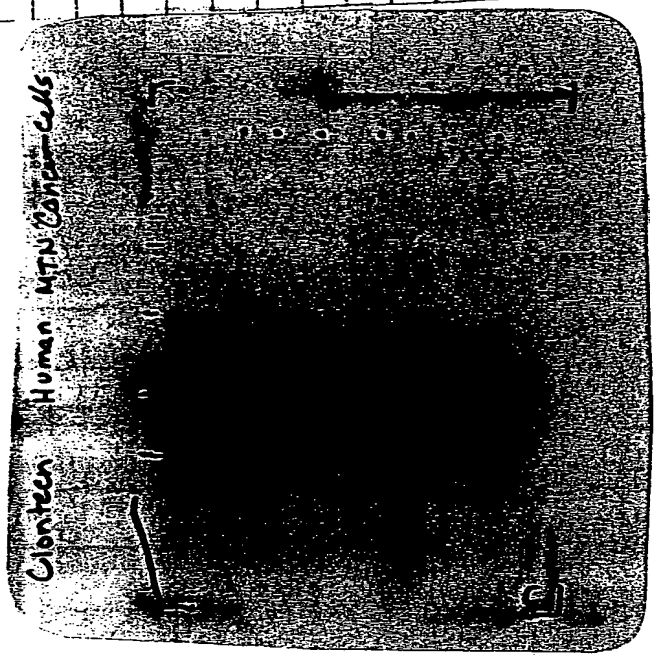
1/2/96 1 Vac Day Off

1/3/96 — 1/5/96 3 Personal Days OFF

1/8/95 — 1/10/96 Company Closed Due to Weather.

1/11/95

Write in the book - Write progress report for Steve

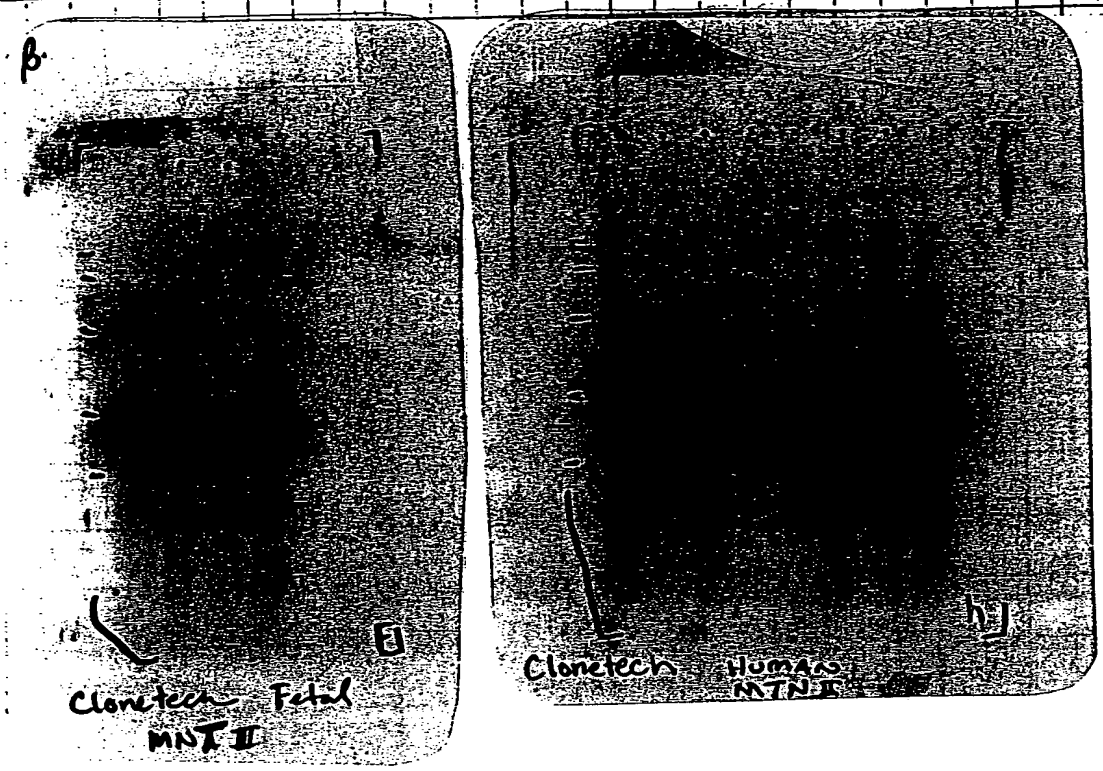


1/3/96 – 1/5/96 3 Personal Days OFF

1/8/96 – 1/10/96 Company Closed Due to weather.

1/11/95

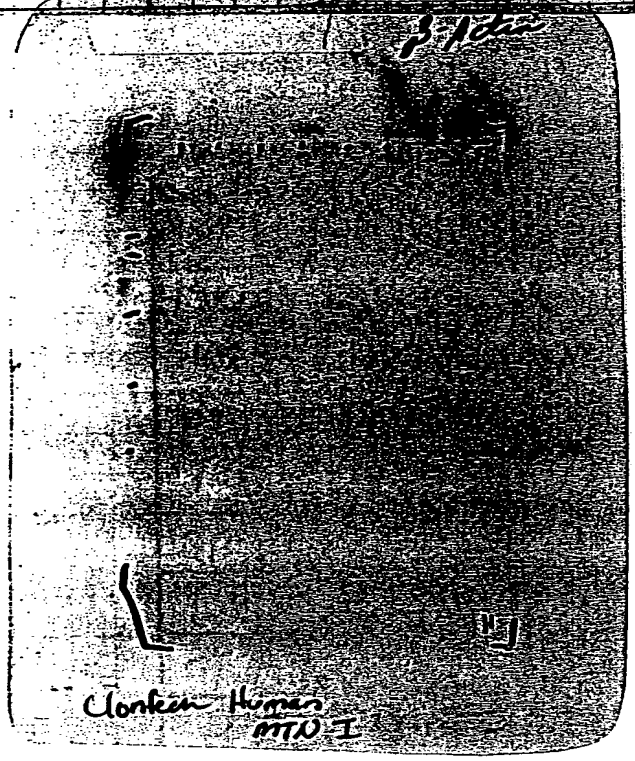
Wrote in note book. Write progress report for Steve



1/3/96 – 1/5/96 3 Personal Days OFF
1/8/96 – 1/10/96 Company Closed Due to Weather

1/1/95

Made in rule book - Write progress report for Steve



1/3/96 – 1/5/96 3 Personal Days OFF

1/8/96 – 1/10/96 Company Closed Due to Weather.

1/11/95

Wrote in note book. Write progress report for Steve

1/12/96 Company Closed due to Weather

12/15/95

1/15/96.

Transform Ligations

for all PDET ligations transform into Chemically Competent MIS rep4 cells and plate onto LB + Amp Kan plates

For all BPPA2 ligations Transform into Chemically Competent DH5α - (GIBCO BRL) cells and plate onto LB + Amp Plates.

Incubate 10 µl of ligation reaction with 100 µl of Competent cells
Incubate on ice 1 hr
Heat 42°C 1 min
Place on ice
Add 700 µl LB
Incubate ~~on ice~~ at 37°C for 1 hr
Plate onto appropriate plates
Incubate 37°C overnight

1/25/96

Set up TNT Reactions T3.

1 HHFFD13
2 HETAQ37
3 HE20I425 15#
4 HTBB4/N515 (+) Control
5 (-) Control H_2O only

	1	2	3	4	5
Rabbit Ret Lup	12.5	12.5	12.5	12.5	12.5
T3 Buffer	1	1	1	1	1
AA ⊖ met	0.5	0.5	0.5	0.5	0.5
35S met	2	2	2	2	2
RNasin	0.5	0.5	0.5	0.5	0.5
T3 Polymerase	0.5	0.5	0.5	0.5	0.5
DNA	0.5	0.5	4	4	0
H_2O	7.5	7.5	4	4	8
	25	25	25	25	25

Set up Reactions on ice

Incubate T3 & T7 Reactions at 30°C 2 hrs.

Spin 1 min
to 5 µl 2X Dissociation Buffer add 5 µl. Rxn
Heat 70°C 5 min.
Quick Spin
Rem on gel at 150V 1½ hrs.

Stop run before Dye front Runs off gel.

Fix for 30 min at 37°C with
Fixative: 10% OHAc
30% Methanol

Dry on BioRad Gel Drier between Cellophane

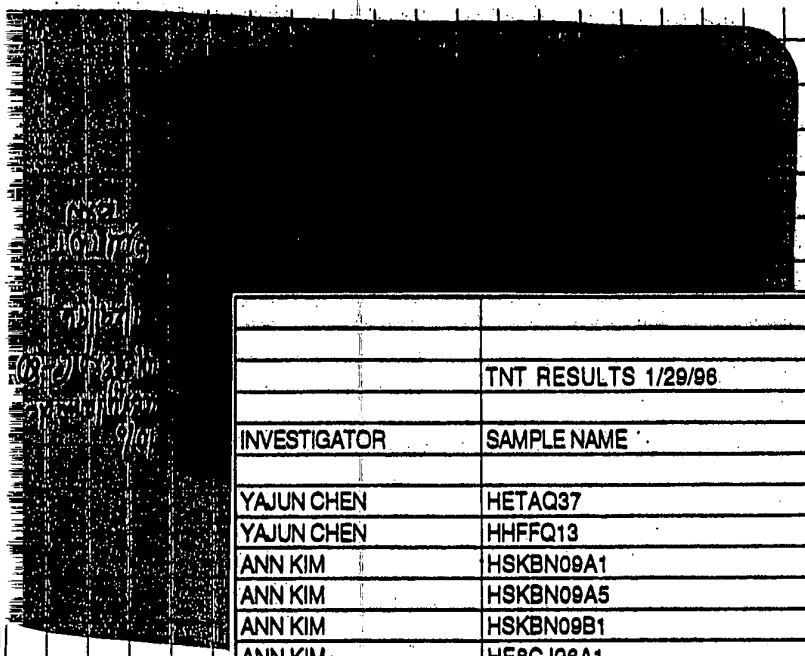
1/25/96

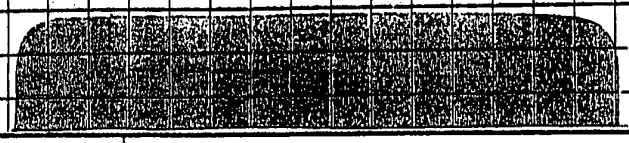
Meeting w/ Henrik Olsen
Laura Orscel
Mario Cepeda
Carol Fischer
Steve Ruben

Discuss Closing Core facility
SOP etc.
Submitted: Closing Request
form for approval of Group.
Decided on Vectors to have on Stock.

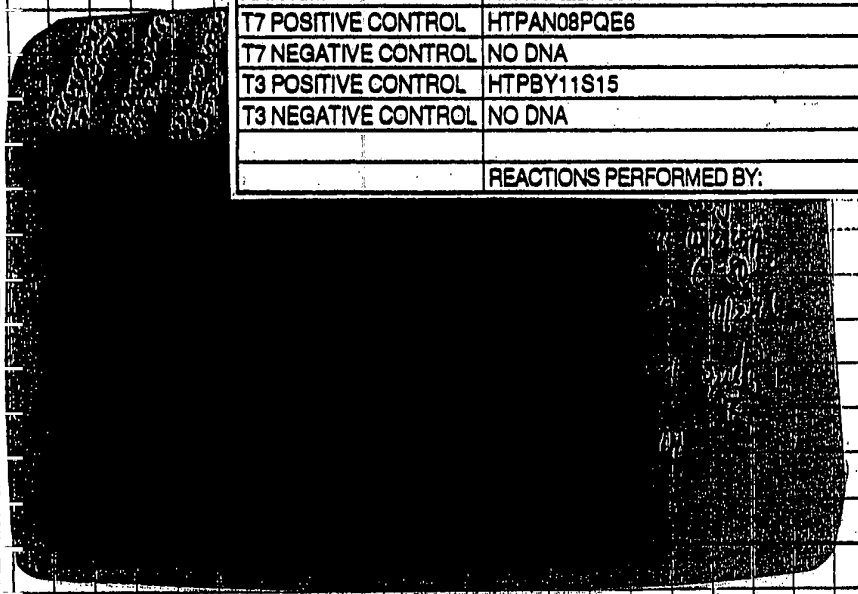
1/26/96

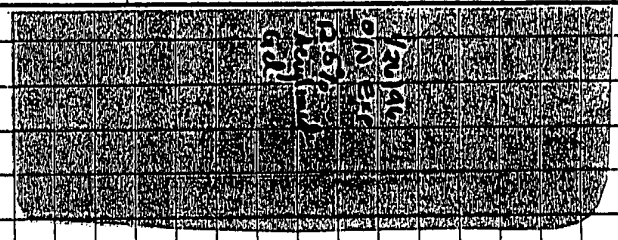
Develop TNT





	TNT RESULTS 1/29/96		
INVESTIGATOR	SAMPLE NAME	EXPECTED PRODUCT SIZE KDa	OBSERVED PRODUCT SIZE KDa
YAJUN CHEN	HETAQ37	51	30 (51 - background?)
YAJUN CHEN	HHFFQ13	110	NO PRODUCT OBSERVED
ANN KIM	HSKBN09A1	37	26, 33, 37
ANN KIM	HSKBN09A5	37	26, 37
ANN KIM	HSKBN09B1	37	NO PRODUCT OBSERVED
ANN KIM	HE8CJ26A1	33	33
ANN KIM	HE8CJ26A5	33	37
ANN KIM	HMSAF22C1	37	33
ANN KIM	HMSAF22D1	37	26, 33, 37
ANN KIM	HMSAF22DELTA3'ASPA2	33	37
T7 POSITIVE CONTROL	HTPAN08PQE6	33	33
T7 NEGATIVE CONTROL	NO DNA	NONE	NONE
T3 POSITIVE CONTROL	HTPBY11S15	94	96
T3 NEGATIVE CONTROL	NO DNA	NONE	NONE
	REACTIONS PERFORMED BY:	ANN KIM	



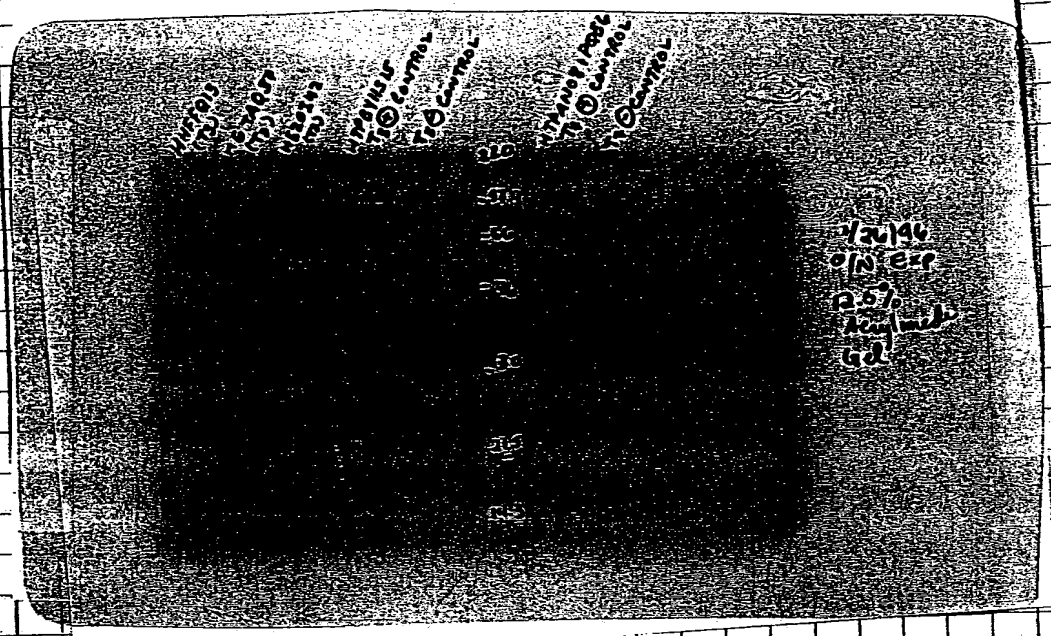


1/29/96

1/29/96

149

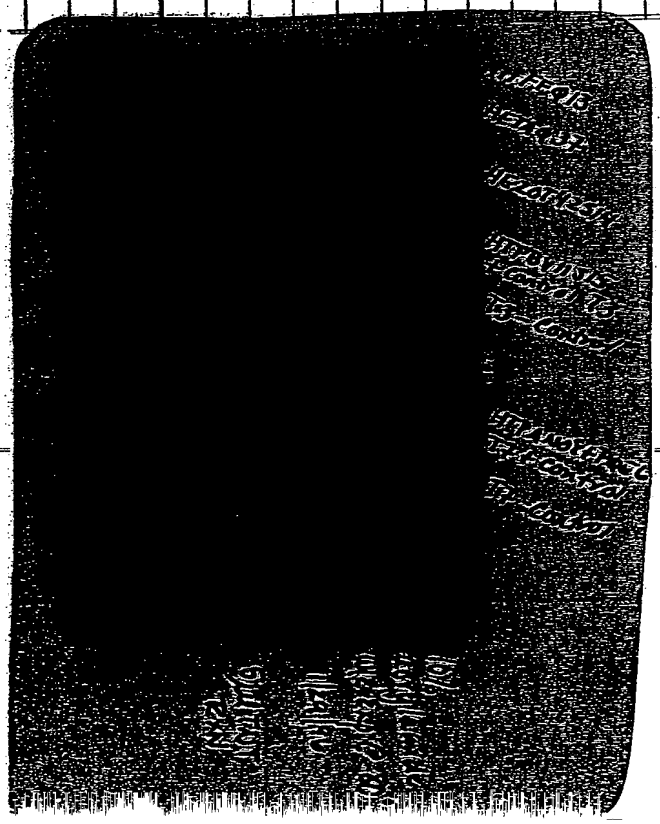
1/24/96

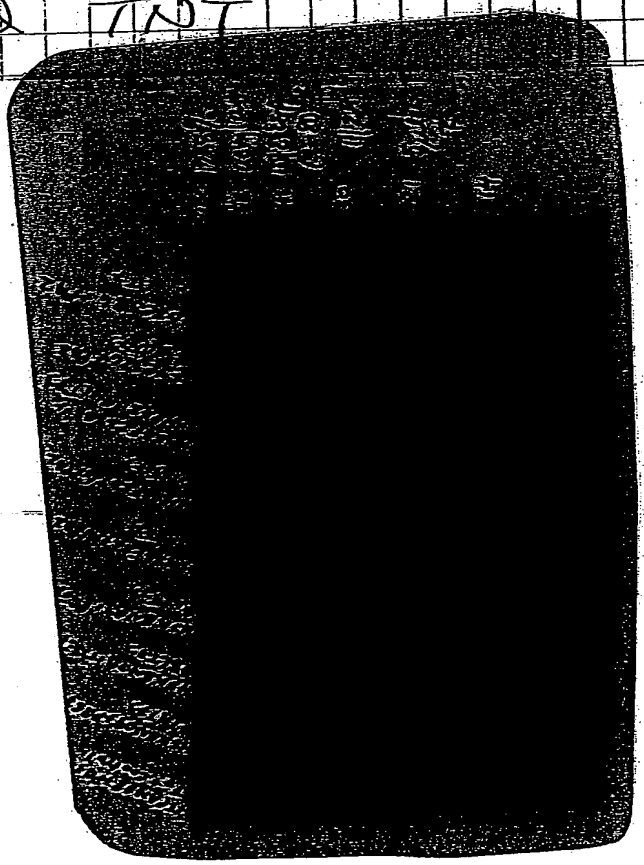


Put on film Longer.

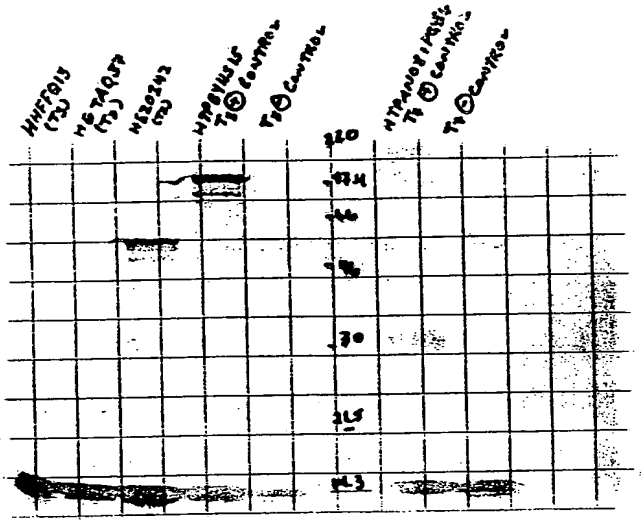
1/29/96.

Carrie Developed TNT





1/26/96

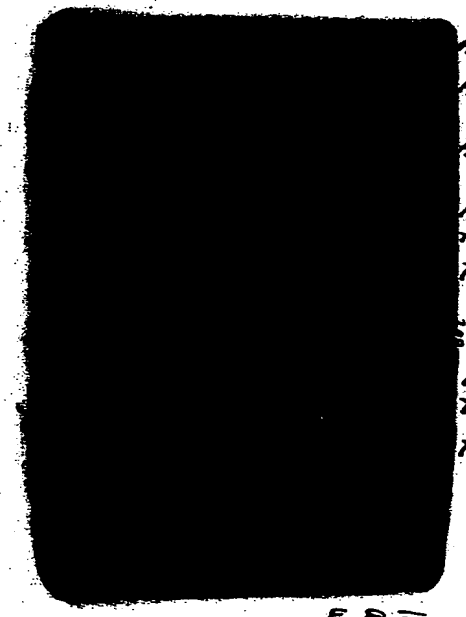


1/26/96
O/N Exp
12.5% Acrylamide Gel

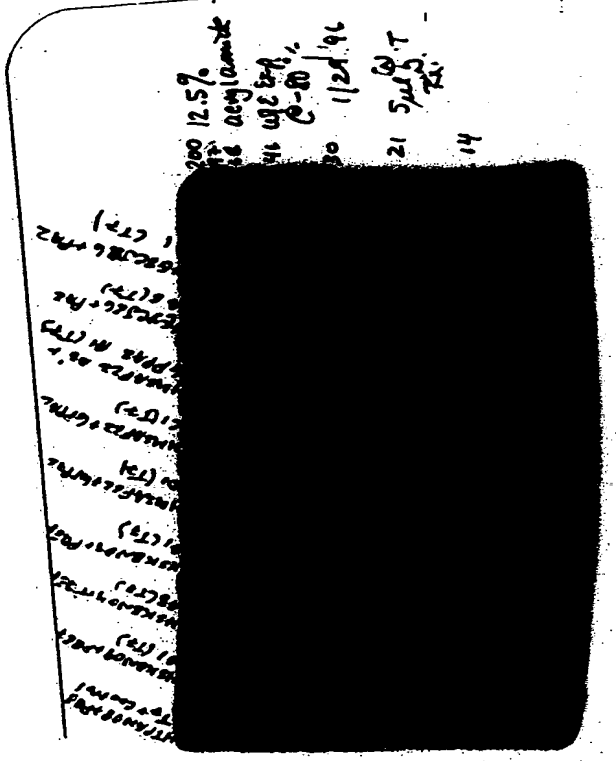
Put on film longer.

1/29/96.

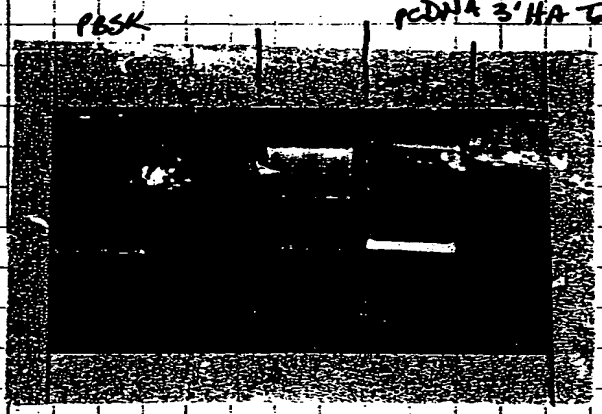
Carrie Developed TNT



10% acrylamide wt Exp @ RT 1/26/96 5µl TNT RW



1/26/96



Store Gel Slices at -20°C Till Return from Vacation

Vacation - 1/29/96 → 2/2/96

1/29/96

Carrie -
Develop Northern